

# SCIENCE

MAY 22, 1953

VOLUME 117

NUMBER 3047

## Contents

- Carbon Isotope Effects in Biological Systems: *Donald L. Buchanan, Akira Nakao, and George Edwards* ..... 541
- Forest Ray Moulton: 1872-1952: *A. J. Carlson* ..... 545

## News and Notes

- National Entomological Societies Merge ..... 546

## Technical Papers

- Effect of Nuclear Counting Procedures on Determination of the Desoxyribonucleic Acid Content of Rat Liver Cell Nuclei: *S. Albert, Ralph M. Johnson, and Renée R. Wagshal* ..... 551
- Biosynthesis of  $C^{14}$ -Specifically Labeled Cotton Cellulose: *Glenn A. Greathouse* ..... 553
- A Cage for Rearing Predator-Prey Populations of Mites: *Thomas Burnett* ..... 554
- Growth and Regeneration in *Hevea* Seedlings: *T. J. Muzik* ..... 555
- The Nature of Perceptual Processes: *Rayford T. Sancer* ..... 556
- Inhibition of Tobacco Mosaic Virus Biosynthesis: *Frank L. Mercer, Taylor E. Lindhorst, and Barry Commoner* ..... 558
- A Snail Intermediate Host of the Rabbit Parasite *Hashtilesia tricolor* (Trematoda: Brachylaemidae): *William B. Rowan* ..... 559
- Polarograms of Oxygen in Lake Water: *K. G. Wood* ..... 560
- Relationship of Colloids to the Surface Tension of Urine: *Robert A. Ravich* ..... 561
- Production of Folinic Acid from Folic Acid by *Lactobacillus casei*: *T. J. Bond* ..... 563
- The Life History of *Echinoparyphium flexum* (Linton 1892) (Dietz 1910) (Trematoda: Echinostomidae): *Haig H. Najarian* ..... 564
- The Cultivation of Hydra Under Controlled Conditions: *W. F. Loomis* ..... 565
- An Ultrafilterable Growth Factor for Tissue Culture: *Sheldon Rosenberg and Paul L. Kirk* ..... 566

## Comments and Communications

- John F. Codington; J. R. King; and Anton Postl* ..... 567

## Book Reviews

- The Forest Insect Problem* ..... 3

## Meetings & Conferences

- ..... 10

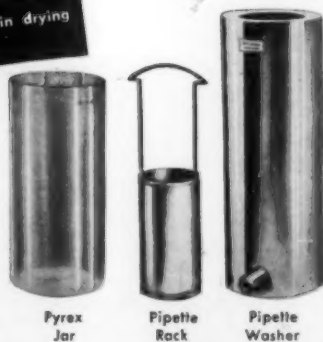
AMERICAN ASSOCIATION FOR THE  
ADVANCEMENT OF SCIENCE



**have lots of  
clean pipettes  
always on hand  
*without drudgery***

As you can see above, it's no problem at all to have plenty of clean pipettes always available — when you entrust the job to the Technicon Automatic Pipette Washer. Twice every minute the washer swirls seven surging gallons of water through the pipettes inside, flushing away clinging residues. In three minutes (six syphon-cycles) it delivers a full basket-load of immaculate pipettes (holds up to 200 pipettes 1 ml and up to 18" long). The special alloy stainless steel basket is proof against corrosive reagents, even potassium dichromate and sulfuric acid. It carries pipettes as a unit-load through cleansing, washing, drying. Requiring no handling of separate pipettes, it practically eliminates breakage and chipping. Bulletin #6150 gives particulars — let us send it to you.

THE TECHNICON COMPANY, 215 East 149 Street, New York 51, N. Y.



**technicon**  
automatic pipette washer

# *Another New Item!*

## **Bird CRYO-DESICCATOR**



Catalog #9-99 ..... Price \$1450.00

### **FURNISHED WITH THE FOLLOWING ACCESSORIES:**

- 1 extra glass tube for tissue heater
- 1 electric cord
- 1 flexible metal tube for attaching to pump
- 1 dial thermometer, Weston 0-180 Deg. F.

*For further information write:*

2385 RHODE ISLAND AVE., N.E.

WASHINGTON, D. C.

*Phipps & Bird*  
INC

6th & Byrd St.  
RICHMOND  
VIRGINIA

Manufacturers and Distributors of Scientific Equipment

# Now! YOU CAN OBTAIN from 1 source

## ALL THE CHEMICALS NORMALLY USED IN THE LABORATORY

With the recent consolidation of the organic division of The Matheson Company and The Coleman and Bell Company, now, for the first time, one company supplies AND GUARANTEES a complete line of 3911 reagent inorganic and organic chemicals, biological stains, solutions and chemical indicators.

Our new 8½" x 11" catalog of over 100 pages will show you that every one of the chemicals in this complete list has been tested by one of our analytical chemists. Where ACS has set up specifications, these are met and all of the more widely used chemicals have either an ACS analysis or minimum specifications.

analysis on the label. A technical grade may have been checked only as to identity . . . while a Reagent Grade with an analysis may have been subjected to fifteen different tests . . . BUT EACH IS GUARANTEED TO MEET THE SPECIFICATIONS LISTED IN THE CATALOG! All of the biological stains in common use have been tested and passed by the Biological Stain Commission.

We have these chemicals in stock, for prompt delivery, at our East Rutherford and Norwood plants. Matheson, Coleman & Bell chemicals are available either through your laboratory supply dealer or from the East Rutherford or Norwood offices.

We shall welcome your request for a copy of this new catalog . . .

**Matheson, Coleman & Bell Products are Distributed  
By Laboratory Supply Houses throughout the World**



# MATHESON, COLEMAN & BELL, INC.



Formerly the organic division of The Matheson Company . . .  
and . . . The Coleman & Bell Company

*Manufacturing  
Chemists*

EAST RUTHERFORD, NEW JERSEY  
NORWOOD (CINCINNATI), OHIO

The Complete  
List of  
**REAGENT  
INORGANIC  
AND  
ORGANIC  
CHEMICALS,  
STAINS,  
SOLUTIONS**

The  
O  
years  
valuab  
or ina  
and t  
operat  
stands  
growing  
the ge  
resour  
Inse  
agents  
injuri  
more  
beetle  
able E  
lion a  
worm  
by air  
Extens  
gon re  
last y  
bids f  
est-ins  
trolled  
ern pi  
heavy  
nume  
forests  
stagge  
Res  
partm  
of for  
of For

DICTION  
metall  
AW., La  
Lansing  
sum for  
24, 1923  
All co  
I.W., W  
K. H. H.





Ruth C. Christman  
Acting Executive Editor

**AAAS EDITORIAL BOARD**  
(Terms expire June 30, 1953)

Bentley Glass, Acting Chairman

|   |                     |
|---|---------------------|
| Mark H. Adams                             | Karl Lark-Horovitz  |
| William R. Amberson                       | Edwin M. Lerner     |
| Wallace R. Brode                          | Walter J. Nickerson |
| F. A. Moulton, Advertising Representative |                     |

## The Forest Insect Problem

ONE of the most important natural resources in this country is its forests. During the last few years wood products of all kinds have become more valuable than ever. Stands long considered too remote or inaccessible for exploitation have been opened up, and tree species hitherto bypassed in commercial operations are being used. The value of commercial stands of timber has also increased. Thus there is a growing recognition on the part of timber owners and the general public of the need for protection of these resources from destructive agents of all kinds.

Insects rank high on the list of destructive forest agents. Fires are more spectacular but hardly more injurious. During the past decade insects have been more troublesome than ever. The Engelmann spruce beetle has killed almost four billion board feet of valuable Engelmann spruce in Colorado. Nearly three million acres of fir timber infested with the spruce budworm in Oregon and Washington have been sprayed by airplanes to prevent wholesale killing of trees. Extensive blow-downs of Douglas fir in western Oregon resulted in an outbreak of the Douglas fir beetle last year. This outbreak is continuing to develop and bids fair to become one of the most destructive forest-insect epidemics ever recorded before it is controlled or subsides. Extensive outbreaks of the southern pine beetle in Texas and Mississippi have caused heavy losses of pines. Other smaller outbreaks too numerous to mention have occurred throughout the forests of the country. The combined losses have been staggering.

Research and surveys conducted by the U. S. Department of Agriculture and leading to the control of forest insects, are a responsibility of the Division of Forest Insect Investigations, Bureau of Entomology

and Plant Quarantine. The Division was established about 50 years ago and during the period of its existence has learned much about our major forest-insect enemies, and has obtained considerable information on the life histories, habits, and artificial control of many of them. The Division carries on its work in 12 field stations located in the major forest regions of the country. It employs about 80 forest entomologists. Half of these men are engaged in research, the remainder in survey activities.

Accomplishments in forest-insect research have been outstanding since World War II. Airplane application of as little as 1 pound of DDT in a gallon of oil per acre of forest has been found effective in controlling most defoliating insects. Costs have been so reduced that this work can be done for about a dollar an acre in large-scale operations. Control of bark beetles and wood borers, insects not amenable to control by airplane spraying, has been improved through the use of new insecticides and better methods of application. A way to prevent losses caused by the western pine beetle in certain stands of ponderosa pine in the West has been perfected. This method of control can be conducted at a profit by land-managing agencies and represents a long step forward on the road to the goal of all forest entomological research—namely, control through prevention of attack.

Preventive methods of control will be stressed in future research. However, direct control measures for the suppression of outbreaks will still be needed for many years to come. Therefore, studies will also be continued to develop cheaper and more effective methods of this nature.

W. L. BAKER

*Bureau of Entomology and Plant Quarantine  
Agricultural Research Administration  
U. S. Department of Agriculture*

SCIENCE, founded in 1880, is published each Friday by the American Association for the Advancement of Science at the Business Press, 10 McGovern St., Lancaster, Pa. Entered as second-class matter at the Post Office at Lancaster, Pa., January 13, 1948, under the Act of March 3, 1879. Acceptance for mailing at the special rate postage provided for in the Act of February 26, 1925, embodied in Paragraph (6-2) Section 34.40 P. L. & R. of 1948. All correspondence should be sent to SCIENCE, 1515 Massachusetts Ave., N.W., Washington 5, D. C. The AAAS assumes no responsibility for the safety of manuscripts or for the opinions expressed by contributors. Four weeks' notice

is required for change of address, and an address stencil label from a recent issue must be furnished. Claims for a missing number will be allowed only if received within 60 days from date of issue.

Annual subscriptions, \$7.50; single copies, \$.25; foreign postage, outside the Pan-American Union, \$1.00; Canadian postage, \$.50. Special rates to members of the AAAS.

The AAAS also publishes THE SCIENTIFIC MONTHLY. Subscription and advertising rates on request.

Cable address: ADVANCESCI.

Reg. U. S.

**GBI**

Pat. Off.

## VITAMINS For Research

Carotene, 90% Beta-10% Alpha

Carotene, Alpha

Carotene, Beta

Vitamin A Alcohol

Vitamin A Acetate, Synthetic

Vitamin A Palmitate, Synthetic

Vitamin B<sub>12</sub> CrystallineVitamin B<sub>12</sub> (Sal. Sol.)Vitamin B<sub>12</sub>-MannitolVitamin D<sub>2</sub>

Acetyl-beta-Methyl Choline Chloride

Acetyl Choline Chloride

p-Aminobenzoic Acid

Ascorbic Acid

Biotin, Crystalline

Biotin Solution

Calciferol, Vitamin D<sub>3</sub>

Calcium Pantothenate

Choline Chloride

DL Desthiobiotin

Folic Acid

i-Inositol

2-Methyl Naphthoquinone (Menadione)

Niacin (Nicotinic Acid)

Niacinamide (Nicotinamide)

Pyridoxine Hydrochloride (Vit. B<sub>6</sub>)

Riboflavin

Rutin

Thiamine Hydrochloride

Tocopherol, Alpha

### A READY REFERENCE

That will Save Time for You

Use this catalog as a "one stop" source of Amino Acids, Vitamins, Carbohydrates, Adenylates, Nucleates, Purines, Pyrimidines, Tetrazolium Salts, Enzymes, Microbiological and Bacteriological Media, Complete Animal Test Diets and Ingredients for investigational use.

**GENERAL BIOCHEMICALS, INC.**  
60 LABORATORY PARK • CHAGRIN FALLS, OHIO



WRITE FOR  
YOUR COPY  
TODAY

## PROTHROMBIN TIME

NOW DETERMINED SIMPLY,  
RAPIDLY, ACCURATELY



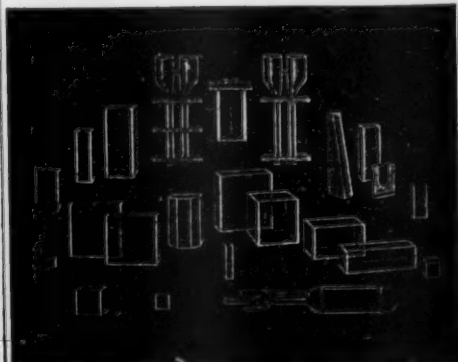
An important, new scientifically designed apparatus that determines PROTHROMBIN TIME accurately and simply. It is a completely integrated unit that eliminates variables in temperature and results. The apparatus is thermostatically controlled at 37°-38°C. and includes an electronically operated stop watch actuated by a foot switch.

For full details write to:

**J. BEEBER CO., Inc.**

838 Broadway, N.Y.C. AL. 4-3510  
1109 Walnut Street, Philadelphia

## GLASS ABSORPTION CELLS made by KLETT



Makers of Complete Electrophoresis Apparatus

SCIENTIFIC APPARATUS

Klett-Summerson Photoelectric Colorimeters—  
Colorimeters—Nephelometers—Fluorimeters—  
Bio-Colorimeters—Comparators—Glass Stand-  
ards—Klett Reagents.

**Klett Manufacturing Co.**  
170 East 87 Street, New York, New York

IT'S  
STANDARD  
PROCEDURE  
to make  
STANDARD  
Your Source  
of Supply

## STANDARD'S SPECIALS of the MONTH!

Keeping Pace with your Laboratory Needs!

### ROTOSTIR

JUST LIKE HAND-STIRRING  
... BUT BETTER!

1. The only stirrer which uses simply a straight piece of glass or metal rod—complicated paddles, propellers, etc. are unnecessary.
2. The only stirrer which permits operation in sealed systems and at the same time leaves the flask bottom unobscured and free for flask heating purposes. It is ideal for use in vacuum distillations, reflux reactions, catalytic hydrogenations, moisture determinations, etc.
3. The only stirrer placed on the market for many years past, utilizing a new and novel operating action—the eccentric drive.
4. The only simple laboratory stirrer adequate for the agitation of viscous liquids and still thoroughly efficient for fluid liquids and two-phase mixing operations.
5. The only stirrer that utilizes the entire submerged area of the stirring rod in the mixing or stirring operation.

No. 93690—Complete, with one eccentric driving disk, one aluminum collar and one each sizes 6, 7, 8 and 9 machined rubber stoppers .... each, \$8.95



### STANDARD LABORATORY CART

"A Real Labor-Saver"

**STAINLESS STEEL CONSTRUCTION**—All-stainless steel products clean instantly and stay gleaming bright. Design is attractive and modern.

**RUGGED DESIGN**—Soundly engineered for years of service and satisfaction. Modern electronic welding assembly methods insure maximum rigidity. All corners and edges rounded smooth. Shelf edges double folded for extra strength.

**NOISEPROOF TREATED**—All shelves have insulating sound deadener. Greatly reduces rattle and clatter of objects placed on shelves.



No. 84975

15½" x 24"

30½"

15½" x 27" x 31½"

22 Gage

20 Gage

200 Pounds

28 Pounds

3" Dia.

\$28.50

No. 84976

17½" x 27"

31½"

17½" x 30" x 32½"

20 Gage

20 Gage

200 Pounds

35 Pounds

3" Dia.

\$35.00

Size of Top and Shelves

Height of Surface of Top

Over-All Dimensions

Solid Stainless Steel in Top

Solid Stainless Steel in Uprights

Carrying Capacity

Shipping Weight

Caster Wheels

PRICE, each

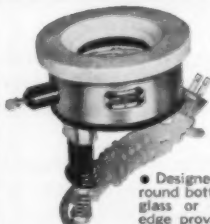
### TYGON "Y" FITTINGS

For Multiple Tubing Connections  
and Branching Lines

Molded in one piece—no seams • Non-toxic—non-contaminating • Does away with glass or metal inserts • Tough, flexible, easy to use • Makes the line ALL TYGON • No flow restriction



|            | EACH<br>(Lots of 5<br>or Less) | EACH<br>(In Lots<br>of 10) |
|------------|--------------------------------|----------------------------|
| No. 87205  |                                |                            |
| 3/16" I.D. | \$ .65                         | \$ .55                     |
| ¼" I.D.    | .65                            | .55                        |
| ⅜" I.D.    | .70                            | .60                        |
| ½" I.D.    | .80                            | .70                        |
| 5/8" I.D.  | .95                            | .85                        |
| ¾" I.D.    | 1.30                           | 1.20                       |
| 1" I.D.    | 1.80                           | 1.70                       |
| 1¼" I.D.   | 2.50                           | 2.40                       |



### STANDARD FLASK HEATER

FEATURES AND  
SPECIFICATIONS

• Designed for heating both beakers and round bottom flasks • Transite top protects glass or non-metallic flasks. Bevel inner edge provides firm support. Easy to install. Clearance hole in boss fits ⅜" laboratory support rod with sturdy thumbscrew adjustment. Rugged all-steel construction.

**SIZE**—Top diameter 4½", with beveled 3" opening on inner edge. Height 2".

**CURRENT**—450 watts at 115 volts AC.

**FINISH**—Body is attractive, and corrosion resisting stainless steel.

**EQUIPMENT**—6 foot Underwriters' Laboratories approved asbestos covered cord and plug.

No. 64355 ..... **\$13.25**

## STANDARD SCIENTIFIC SUPPLY CORP.

34 West 4th Street • New York 12, N. Y.

LABORATORY APPARATUS—REAGENTS AND CHEMICALS



Ready June 10th: First book on the applications of X-ray methods to the problems of organic chemistry

# Organic Crystals and Molecules

*Theory of  
X-Ray Structure Analysis  
with Applications to  
Organic Chemistry*

BY J. Monteath Robertson, HEAD OF THE  
DEPARTMENT OF PHYSICAL CHEMISTRY,  
THE UNIVERSITY OF GLASGOW

Only within the last twenty years have the methods of modern crystallography become sufficiently refined to deal with the structures of organic crystals in detail, and still more recently have they been extended to embrace the even more complex structures of direct interest to biochemistry.

Here the principles of the science of X-ray analysis are clearly set out, with a full account of the most recent developments, their possibilities and their limitations.

Applications to organic chemistry are discussed in terms of bond length measurement and the determination of molecular geometry; the study of molecular arrangement, hydrogen bonded structures, and molecular compounds; the elucidation of complex and chemically unknown structures; and finally, applications to macromolecular and biological materials are considered.

132 illustrations, 360 pages (approx.) \$5.00

**Cornell University Press**

124 ROBERTS PLACE, ITHACA, NEW YORK

*Now available...*

Adenosine Triphosphate (ATP); Amygdalin; Amylase; Animal Lecithin; Ascorbic Acid Oxidase; Bacitracin; BAL; Biotin, cryst.; Caffeic Acid; Carotene, cryst.; Catalase; Cellulase Chlorogenic Acid; Chorionic Gonadotropin; Circulatory Hormone; Colchicine; Cytidylic Acid; Cytochrome C; Dehydroascorbic Acid; Diacetoneamine; Dihydroxyacetone; Dihydroxyphenylalanine (DOPA); Dipyriddy; Edestin; Emulsin; Erythritol; N-Ethylpiperidine; Fibroin; Folic Acid; Galacturonic Acid; Gentisic Acid; Girard Reagents P and T; Gliadin; Glucose Pentaacetate; Glucuronic Acid; Glyceraldehyde; Glyceric Acid; Heparin; Hordenine; Hyaluronidase; Hydrindene; 2-Hydroxyadipaldehyde; Humulon; Indan; Isoascorbic Acid; Isopropylarterenol; Kojic Acid; Kynurenic Acid; Lanthionine; Lipase; Lysozyme; Lyxose; Malononitrile; Maltase; Melezitose; Mesobilirubinogen; Muscle Adenylic Acid; p-Nitrophenylphosphate; Nucleoprotein; Orcinol; Pancreatin; Pantothentic Alcohol; Penicillinase; Peroxidase; Phenazine; Phenylpyruvic Acid; Phloridin; Phosphorylase; Piperin; Porphyrindine; Protamines; Protoporphyrin; Pyridoxal; Pyridoxamine; Pyrocatechuic Acid; Pyruvic Aldehyde; Ribonuclease; Saccharic Acid; Salmine; Serine Phosphoric Acid; Spermidine; Spermine; Thioacetic Acid; Thiocytosine; Thyroxine; Trigonelline; Triphenyltetrazolium Chloride; Tripyridyl; Trypsinogen; Tyrosinase; Tyrothricin; Urease; Uracase; Uridine; Vitellin; Xanthosine.

Ask us for others!

**DELTA CHEMICAL WORKS**

23 West 60th St. New York 23, N.Y.  
Telephone PLaza 7-6317

## UNITRON PHASE MICROSCOPES

Complete phase contrast microscopes, of revolutionary new design, priced so low that every laboratory can now afford to use the powerful methods of phase microscopy.

Indispensable for the study of living cells, tissues, bacteria, microorganisms and other material too transparent to be seen with ordinary bright field microscopes. Eliminates the need for staining. Many industrial, as well as biological, applications.

- Can also be used for ordinary bright field microscopy. Model MPE allows continuous transition from bright field to phase contrast.
- Extremely simple to use. No diaphragm adjustments.
- Highest revolving power is obtained.
- No special illumination required.
- Choice of 4 contrasts: Bright-high, medium and low, Dark-low.



**MODEL MPE:** Magnification 50-1500X. Large research stand with coarse and fine focusing, graduated drawtube, mechanical stage. Back and pinion substage phase condenser. Triple revolving nosepiece with phase objectives: 10X, 40X 100X (oil immersion). Eyepieces: 5X, 10X, 15X.

**\$265**

**MODEL MPEA:** Magnification 32-600X. Medium stand with single focusing control. Substage phase diaphragm. Triple revolving nosepiece with phase objectives: 4X, 10X 40X. Eyepieces: 8X, 15X.

**\$99**

Model MPE is available equipped for bright field observation only at a cost of \$198. All microscopes furnished with wooden cabinets and accessories. Shipped express collect.

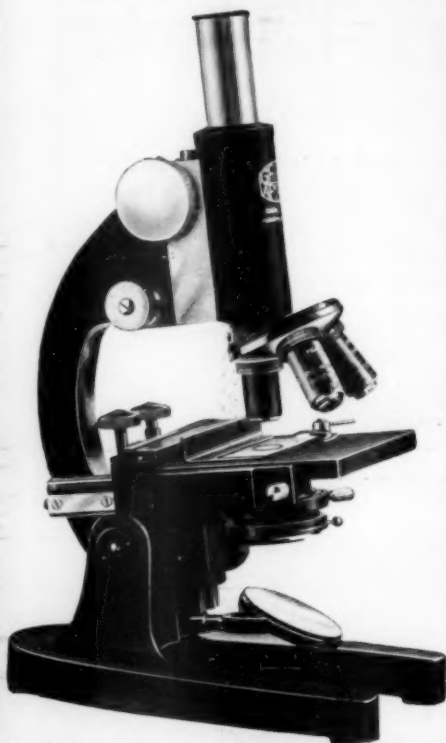
**ALL INSTRUMENTS FULLY GUARANTEED**

Institutions and rated firms shipped on open account; others send check with order or 25% deposit, with balance C.O.D.

Write for illustrated literature to Dept. R-104

*United Scientific Co.* 204-6 Milk St.  
Boston 9, Mass.

**MEDICAL  
GRAF-APSCO MICROSCOPES**



**MODEL GKM, MEDICAL MICROSCOPE**

Designed to eliminate the usual sources of irritation. For a detailed list of improvements write for descriptive circular.

**QUALITY UNSURPASSED**

**OUR LOW PRICE UNBEATABLE**

Model GKM<sub>s</sub> equipped as follows:

- 5x and 10x Huyghenian Oculars
- Achromatic Objectives:
- 16 mm (10x); 4 mm (44x) and
- 1.8 mm (100x) oil immersion
- Abbe Condenser with iris diaphragm
- operated by rack and pinion
- GRADUATED Mechanical Stage
- Double (plane/concave) Mirror
- Upright or satchel type case (same price)

**\$255.00** each. Less 10% on 5 or more

Old microscopes accepted in trade.

**THE GRAF-APSCO CO.**

**5868 Broadway**

**Chicago 40, Ill.**

**RESEARCH  
BIOCHEMICALS**  
For  
**INVESTIGATIONAL USE**  
**NUCLEO PROTEINS**  
**PURINES PYRIMIDINES**

ADENINE  
ADENINE SULFATE  
ADENOSINE  
ADENOSINE DIPHOSPHATE  
ADENOSINE TRIPHOSPHATE—93% ATP  
(Sodium and Barium salts)  
ADENYLIC ACID  
(Adenosine 3 phosphoric)  
(Adenosine 5 phosphoric)  
AMMONIUM URIDYLATE  
COZYMASE (COENZYME I)  
CYTIDINE  
CYTIDINE SULFATE  
CYTIDYLIC ACID  
CYTOSINE  
2 THIO CYTOSINE  
DESOXYRIBONUCLEIC ACID  
FRUCTOSE-6-PHOSPHATE  
GLUCOSE-1-PHOSPHATE  
GLUTATHIONE  
GLUCOSE-6-PHOSPHATE  
GUANINE (Free Base)  
GUANINE HYDROCHLORIDE  
GUANOSINE  
GUANYLIC ACID  
HEXOSE DIPHOSPHATE  
(Fructose-1-6-Diphosphate)  
HYPOXANTHINE  
INOSINE  
IRON NUCLEATE  
ISO-CYTOSINE  
MANGANESE NUCLEATE  
5-METHYL THIOURACIL  
6 (-) METHYL URACIL  
5-METHYL URACIL  
NUCLEIC ACID  
(Ribose Nucleic Acid)  
PHOSPHOGLYCERIC ACID (D-3)  
PROTAMINE NUCLEINATE  
PROTAMINE SULFATE  
D (-) RIBOSE  
SODIUM RIBONUCLEATE  
SODIUM DESOXYRIBONUCLEATE  
THYMINE (5 METHYL URACIL)  
URACIL  
URAMIL (5 Amino Barbituric Acid)  
URIDINE  
URIDYLIC ACID  
XANTHINE  
XANTHOSINE

**WRITE FOR  
NEW  
CATALOGUE**

#S 998

Listing over 900

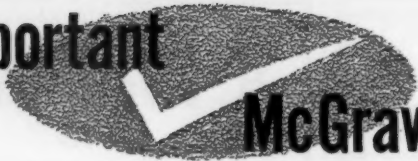
Research Biochemicals



*Nutritional Biochemicals Corporation*  
CLEVELAND 28, OHIO



# Important



# McGraw-Hill Books

## CLAY MINERALOGY

By RALPH E. GRIM, University of Illinois. *McGraw-Hill Series in Geology*. In press

A distinguished text by a world authority based on the increased research activity of the past twenty-five years. The book presents all available data on the atomic structure, composition, physical characteristics, origin, and occurrence of clay minerals. It is carefully prepared, clearly presented, and well illustrated with drawings and tables of data.

## GEOLOGY

By O. D. VONENGELN, Cornell University, and KENNETH E. CASTER, University of Cincinnati. 730 pages, \$7.00

A basic text covering both physical and historical geology, this book starts with geologic phenomena familiar to the student, and, following this consideration of physical geology, presents the facts of historical geology as an outgrowth of physical. A strong narrative continuity is maintained throughout with logical sequences of chapters and topics.

## INVERTEBRATE FOSSILS

By RAYMOND C. MOORE, C. G. LALICKER, and A. G. FISCHER, University of Kansas. 766 pages, \$12.00

A well-organized, comprehensive, descriptive guide to all important fossils, containing more than 400 line drawings, illustrating over 2,000 fossils, this text gives, first, clear accounts of important structural characters, especially of hard parts. It then discusses evolutionary trends. Stratigraphically arranged groups of representative examples are illustrated. Special emphasis is on *graphic presentation* and avoidance of *unnecessary terminology*.

## GUIDE TO GEOLOGIC LITERATURE

By RICHARD M. PEARL, Colorado College. 239 pages, \$4.50

A guidebook to the extensive and complex world-literature of geology, its subdivisions, and its related fields. It includes all published literature in every language, from the earliest dates to current material of vital significance. It is not a subject directory but, rather, a detailed overall guide to the available types of geologic literature.

*Send for copies on approval*



**McGRAW-HILL BOOK COMPANY, INC.**

330 WEST 42ND STREET, NEW YORK 36, N. Y.



# Carbon Isotope Effects in Biological Systems

Donald L. Buchanan,<sup>1</sup> Akira Nakao, and George Edwards<sup>2</sup>

Division of Biological and Medical Research, Argonne National Laboratory,  
Lemont, Illinois, and Institute of Nuclear Studies,  
University of Chicago, Chicago, Illinois

**T**RACER STUDIES are subject to error when labeled and unlabeled molecules differ quantitatively in their chemical and physical properties. Such differences in behavior are related to atomic mass and are called isotope effects. This phenomenon has been subjected to rather rigorous theoretical treatment in the case of simple chemical reactions (1-3). The application of precise physicochemical theory to biological isotope effects is not likely to be so successful, and for this reason most of the existing knowledge in this field has been obtained empirically. Isotope effects have been studied in biological reactions involving hydrogen (4, 5), oxygen (6), nitrogen (7), potassium (8), and carbon (6, 9-14).

In the case of carbon-14, kinetic studies have shown very striking differences in the rates of uptake of  $C^{14}O_2$  and  $C^{13}O_2$  by barley seedlings (13) and algae (13, 14). In the latter work, Weigl found that when a small inoculum of the microscopic plants had, through growth, converted 70 per cent of the available  $CO_2$  to organic material the specific activity of the plant carbon was 24 per cent less than that of the dissolved inorganic carbon. Mass spectrometric analyses for carbon-13 on the same samples showed a difference of only 4 per cent in the same direction. The latter figure is in satisfactory agreement with results of other workers (6, 11, 12) when allowance is made for kinetic effects (*vide infra*), but on theoretical grounds the fractionation factor for carbon-14 is expected to be approximately twice the carbon-13 factor (3, 15).

Other investigators have found lesser biological isotope effects with carbon-14 in steady state systems. In studies on the worldwide distribution of this isotope, Libby and his associates have measured the radioactivity of natural carbon sources (9, 10). They found that specimens of wood had  $7.3 \pm 3.0\%$  per cent less radioactivity on a carbon basis than sea shell carbonate samples. Very recently Kulp, Feely, and Tryon (16) have found modern wood to contain  $9.7 \pm 1.5\%$  per cent less natural radiocarbon than clam shells.

The present work is a brief survey of isotope effects with carbon-14 in biological reactions and, in addition, compares the fractionation factor of carbon-13 with

the carbon-14 factor. An attempt has been made to account for some apparently conflicting results of other workers.

*"Steady state" experiment.* A 25-gallon aquarium was provided with a mercury thermoregulator, a glass cooling coil, and connections of glass tubing, all passing through a Lucite top. Before sealing the tank a 2-inch layer of washed quartz sand was placed uniformly over its bottom, and the fluid from a small open aquarium added to provide microorganisms. Small numbers of several plants, including *Vallisneria* sp. (eel grass), *Ceratophyllum demersum*, and *Lemna minor* (duck weed), were placed in the water, and 5 gallons of a heavy culture of *Scenedesmus obliquus* were added. The medium in which these algae had grown contained  $NaHC^{14}O_3$  as the sole carbon source. After the top had been fastened in place with a cemented gasket and clamps, the tank when tested for leakage held a small positive pressure of air for several hours. Tap water was circulated through the cooling coil, and the thermoregulator, set at  $22^\circ$ , was connected to a relay that activated an incandescent flood lamp. The latter served as a heater as well as an illuminator. A small fluorescent lamp was placed adjacent to the tank as a constant source of light. A few days after the tank was sealed, 2 pairs of guppies and about 100 small planorbid snails (*Helisoma* sp.) were added through one of the glass tubes.

During the first year the water in the aquarium was heavily clouded with green algae, and the guppies became quite numerous. During the second year, as the higher plants grew and multiplied, the algae thinned out and the fish population diminished. During the final year the water remained clear, and *Vallisneria* became the predominant plant. The fish disappeared during the middle of the third year. During the second year a filamentous alga, *Oscillatoria* sp., appeared in the vicinity of the fluorescent lamp. After a heavy growth had accumulated, the lamp was moved to the opposite side of the tank, and the alga grew there and diminished at the first site. The snail population remained relatively constant for the first 2 years, but during the final year tended to diminish in both size and number. Dead snail shells accumulated over the 3-year period, and the algal sediment gradually became darker in color until it was almost black. The foregoing indicates that a continuous turnover of carbon was taking place, but that mixing was probably never complete, some material such as snail shells and organic sediment becoming relatively inaccessible to metabolic turnover.

<sup>1</sup> Present address: Department of Biochemistry, Western Reserve University, Cleveland, Ohio.

<sup>2</sup> The authors wish to thank A. O. Nier for his assistance in confirming the relative abundances of the carbon isotopes, and Harold C. Urey for his advice and criticism of the methods and calculations.

<sup>3</sup> Standard error.

At the end of the third year various samples were removed for analysis. The determinations were carried out by means of proportional gas counting, as described elsewhere (17). To prevent contamination of specimens with air  $\text{CO}_2$ , certain precautions were taken. Water samples were withdrawn through one of the sampling tubes into evacuated flasks before the tank was opened. The other samples were withdrawn with forceps, hooks, and siphons fitted with diaphragms that covered the tank orifice during manipulations. The samples were quickly placed in vials, sealed, and then frozen before drying. In addition to these precautions, tests were conducted to determine whether the specific activity of the plants changed with time when left exposed to room air. No change was noticed in 2 hours, and only a slight diminution was found after 2 days.

Multiple samples of the more abundant organisms were taken separately and analyzed separately. The data obtained are summarized in Table 1. Each of

TABLE 1  
SPECIFIC ACTIVITIES OF SPECIMENS IN AN ISOTOPICALLY  
LABELED AQUARIUM AFTER PROLONGED  
ISOLATION FROM EXTRANEAL  
CARBON SOURCES

| Sample                              | No. of<br>samples<br>as-<br>sayed | Specific Activity   |   |
|-------------------------------------|-----------------------------------|---|---|
|                                     |                                   | (cts/min/mM)  | Relative<br>(% of<br>dissolved<br>$\text{CO}_2$ ) |
| $\text{CO}_2$ dissolved<br>in water | 8                                 | $204,300 \pm 500^*$   | $100 \pm 0.3^*$                                   |
| Plants                              |                                   |   |   |
| <i>Vallisneria</i>                  |                                   |   |   |
| Tops                                | 7                                 | $192,300 \pm 900$   | $94.0 \pm 0.5$                                    |
| Middle leaf<br>sections             | 5                                 | $193,000 \pm 800$   | $94.5 \pm 0.4$                                    |
| Roots                               | 3                                 | $194,600 \pm 400$   | $95.3 \pm 0.2$                                    |
| Stalk bases                         | 4                                 | $196,900 \pm 900$   | $96.4 \pm 0.5$                                    |
| Whole young<br>leaves               | 2                                 | $194,500 \pm 1000$  | $95.2 \pm 0.5$                                    |
| <i>Ceratophyllum</i>                | 1                                 | 198,500   | 97.2  |
| <i>Lemna minor</i>                  | 1                                 | 195,600   | 95.7  |
| <i>Oscillatoria</i>                 | 5                                 | $197,300 \pm 500$   | $96.6 \pm 0.3$                                    |
| <i>Heliozoa</i>                     |                                   |   |   |
| (living snails)                     |                                   |   |   |
| Shell carbonate                     | 8                                 | $208,600 \pm 300$   | $102.1 \pm 0.2$                                   |
| Organic matter                      | 4                                 | $196,100 \pm 1000$  | $96.0 \pm 0.5$                                    |
| Dead snail shells                   |                                   | 220,000†<br>234,100<br>221,100<br>234,400<br>245,600†<br>240,400<br>250,500 |   |
| Organic sediment                    |                                   |   |   |

\* Standard error of mean.

† Separate analyses.

the recorded standard errors was calculated from the results of a replicated series of analyses and was not derived by the application of the statistics of counting, as is customary in reporting radioactivity measurements. The error estimates, therefore, include all

random manipulative error as well as genuine sample variation, if any.

Consistent with all the published data discussed above, these results show that plants tend to reject the heavier isotope in favor of carbon-12. The results with *Vallisneria* show that the heavy stalks at the base of the plants had a slightly higher specific radioactivity than the tops, and the central leaf section was intermediate. This is presumptive evidence that the isotope content of available  $\text{CO}_2$  was slowly diminishing during the growth of these plants. This may have been partly the result of slow turnover in the original unlabeled organisms and partly the result of the acquisition of unlabeled  $\text{CO}_2$  by the system from undetected sources, e.g., leakage or through bacterial action on the aquarium cement or gasket. The analyses on the dead snail shells and organic sediment definitely show that a true steady state had not been attained during the 3-year period. However, it seems likely that the rate of change was very slow at the end of this time, and that a comparison between the  $\text{CO}_2$  in solution and recently growing organisms gives a close estimate of the actual isotope effect in a steady state.

Although plants and the organic matter of snails appeared to reject the heavier isotope, the carbonate of the shell tended to concentrate it to some extent. In living snails the difference in specific activity between the organic material and the shell carbonate was 6 per cent, and the difference between recently grown plant tissue and shell carbonate was about 9 per cent. These differences in isotope concentration are approximately twice those found with carbon-13 (6, 11, 12) in similar materials and are in agreement with the observations of Anderson and Libby (9), and of Kulp and associates (16) on the natural abundance of carbon-14 in material of this type.

None of these data shows as spectacular differences as were found by Weigl and Calvin (13) and Weigl (14). Since this type of discrepancy could have resulted from differences in kinetics, the isotope dynamics in a sealed system of growing algae were studied in a second experiment.

*Isotope kinetics during algal growth.* A 12-gallon Pyrex carboy was fitted with a rubber stopper holding a glass cooling coil, thermoregulator, thermometer, and 3 lengths of glass tubing for sampling. A magnetic stirrer was inserted, and 30 liters of  $\text{C}^{14}$ -labeled inorganic culture medium were added to the bottle. The stopper assembly was tightly wired on the carboy, and the thermoregulator set at  $22^\circ$ . Refrigerated water was circulated through the cooling coil, and 2 reflector flood lamps activated by the thermoregulator were used to maintain the temperature as well as to furnish light. Before algae were added, analyses of the  $\text{CO}_2$  present in solution on 3 successive days showed the mean specific activity to be  $50,530 \pm 10^3$  cts/min/mM of carbon. The medium was then inoculated with 90 mg (dry weight basis) of radioactive *Scenedesmus obliquus*. The specific radioactivity of this inoculum was within 2 per cent of the activity of

the medium, thus minimizing isotope dilution error during early sampling. Samples of the culture were taken at intervals during this period and analyzed for concentration and radioactivity of both  $\text{CO}_2$  and algae. The pH of each sample was measured and 50 ml of  $N$  HCl were added each time the pH rose above 7.0.

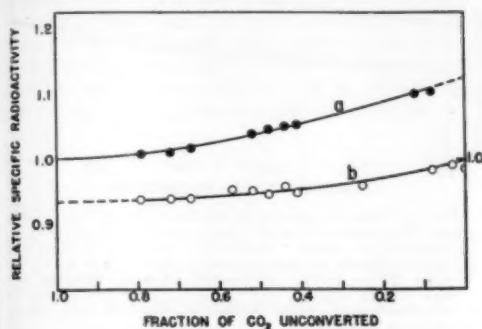


FIG. 1. Change in specific radioactivities of dissolved  $\text{CO}_2$ -bicarbonate (curve a) and of algal carbon (curve b) during growth. (Relative specific radioactivity 1.0 equals 59,530 cts/min/mM of carbon.)

Figure 1 shows the specific radioactivity of inorganic carbon and of algae relative to the initial level as a function of the fraction of  $\text{CO}_2$  unconverted to algal carbon. The first samples, taken after 21 per cent of the total  $\text{CO}_2$  had been converted, showed that the radioactivity in the organic carbon was 7 per cent lower than in dissolved  $\text{CO}_2$  and bicarbonate. At this stage of growth the original inoculum was diluted 30-fold, and its contribution could not have altered the algal specific radioactivity by more than 0.1 per cent. As growth progressed, the difference gradually increased and was 11 per cent when 92 per cent of the  $\text{CO}_2$  had been converted. Allowing for experimental error, the final algal specific radioactivity was equal to that of the  $\text{CO}_2$  initially.

The gradual increase with growth of the difference in isotope content makes it necessary to extrapolate the data to the point where conversion first began in order to estimate the true difference between the conversion rates of  $\text{C}^{14}\text{O}_2$  and unlabeled  $\text{CO}_2$ . A plot of the logarithm of the specific radioactivity ratios of algal to inorganic carbon against the fractional conversion was found to be approximately linear. A regression analysis of these data showed the initial ratio to be  $0.935 \pm 0.005^2$ . This ratio is in satisfactory agreement with the most reliable value of Table 1, that of recently grown *Vallisneria* shoots,  $0.940 \pm 0.005^2$  (relative to dissolved  $\text{CO}_2$ ).

**Comparison of the isotopes effects with carbon-13 and carbon-14.** For this comparison the algal growth experiment was repeated. Samples of  $\text{CO}_2$  and algae were taken on two successive days when more than half of the inorganic carbon had been incorporated into the plants. The samples were converted to  $\text{CO}_2$ , and the gas was divided and analyzed for specific

radioactivity by gas counting (17) and for carbon-13 abundance by mass spectrometry. The mass spectrometer was one of those used by Urey (18) and his group for the measurement of small changes in isotope abundance ratios in which the maximum experimental deviation is within  $\pm 0.01\%$  of the absolute abundance. A correction was applied for the presence of  $\text{C}^{12}\text{O}^{18}\text{O}^{17}$ . For each pair of  $\text{CO}_2$  samples the one which was originally inorganic was used as a standard against which the mass abundance of the algal  $\text{CO}_2$  was measured. To ensure that no systematic error was present in the mass spectrometer measurements, one pair of carbon dioxide samples was referred to A. O. Nier at the University of Minnesota. The absolute abundance values he obtained confirmed the results by the direct difference method.

The results appear in Table 2 and show the ear-

TABLE 2  
COMPARISON OF ISOTOPE EFFECTS WITH  
CARBON-14 AND CARBON-13

| Sample No. | $\text{C}^{14}$ abundance |                     |                               | $\text{C}^{13}$ abundance     | $\text{C}^{14}$ abundance difference |
|------------|---------------------------|---------------------|-------------------------------|-------------------------------|--------------------------------------|
|            | $\text{CO}_2$ e/m/mM      | Algal carbon e/m/mM | Difference % of $\text{CO}_2$ | Difference % of $\text{CO}_2$ | $\text{C}^{18}$ abundance difference |
| 1A         | 73,510                    | 65,730              | 10.6                          | 4.20                          | 2.52                                 |
| 1B         | 73,240                    | 65,990              | 10.2                          | 4.25                          | 2.43                                 |
|            |                           | 66,580              | 9.1                           |                               | 2.14                                 |
| 2A         | 74,030                    | 65,830*             | 10.1                          | 4.39                          | 2.38                                 |
|            |                           | 66,400              | 10.3                          |                               | 2.35                                 |
| 2B         | 74,070                    | 66,160*             | 10.4                          | 4.37                          | 2.37                                 |
|            |                           | 66,320              | 10.5                          |                               | 2.40                                 |
| 2C         | 74,280                    | 65,920              | 11.3                          | 4.46                          | 2.53                                 |
|            |                           | 66,160*             | 10.8                          |                               | 2.42                                 |
| Av         |                           |                     |                               |                               | $2.39 \pm 0.04$                      |

\*  $\text{CO}_2$  absorbed in ethylenediamine and liberated with  $\text{H}_2\text{SO}_4$ .

† Standard error.

bon-14 effect to be distinctly more than double the carbon-13 effect. The mean ratio of the separate isotope effects is  $2.39 \pm 0.04^2$ . The value is not in agreement with the theoretical separation factor of 1.98 based on mass difference alone (2, 15). This deviation might be partially accounted for by the necessarily different methods of preparation of  $\text{CO}_2$  from the inorganic and organic samples, but the analysis of several compounds with this method has given a recovery error much lower than would account for this variation from the theoretical value. Furthermore, absorption of some of the samples in carbonate-free alkali (19) with subsequent evacuation and collection of neutral impurities gave entirely negative results ( $< 0.1\%$ ). Subsequent acidification and collection of the gas evolved gave  $\text{CO}_2$  with no significant change in activity except in sample 1B, where the second analy-

sis was lower and more consistent with its duplicate 1A. This deviation from the theoretical value has been noted recently by Stevens *et al.* (20), who have carried out experiments specifically designed to evaluate this factor. These workers chose the partial decarboxylation of mesitoic acid as a fractionating system and obtained a value of  $2.66 \pm 0.15^4$  for the ratio. In this work both isotopes were measured mass spectrometrically.

It is implied in the discussion by Bigeleisen (1) that the apparent isotope effect at any stage of a reaction is influenced by the degree of reversibility. In biological reactions such as the ones studied here the extent of reversibility, a quantity difficult to estimate, probably depends to some extent upon the overall rate of the process. Since the rate may vary with the experimental conditions, separate experiments may give different results. A theoretical consideration of the algae experiment allows an evaluation of the extent to which reversibility could have influenced the data. To simplify the mathematical consideration the conversion of inorganic to algal carbon will first be regarded as irreversible and then as completely reversible.

The irreversible conversion is satisfactorily dealt with by the Rayleigh equation (21), originally applied to batch distillations. As employed in the present situation the equation may be written:

$$\ln C = \int_{x_0}^x \frac{dx}{y-x}, \quad (1)$$

where  $C$  is the fraction of the original  $\text{CO}_2$  that remains in the inorganic form,  $x$  is the specific radioactivity of the  $\text{CO}_2$ , and  $y$  is the specific radioactivity of carbon as it enters the algae. Because the mole fraction of  $\text{C}^{14}\text{O}_2$  remains exceedingly low, the radioactivity of carbon transferred to the algae may be represented by the equation:

$$y = bx, \quad (2)$$

where  $b$  is a constant. Substituting in equation (1) and integrating:

$$\ln C = \frac{1}{b-1} \ln \frac{x}{x_0}; \quad (3)$$

$$\frac{x}{x_0} = C^{b-1}. \quad (4)$$

By material balance:

$$\bar{y}(1-C) + xC = x_0, \quad (5)$$

where  $\bar{y}$  is the specific radioactivity of the total algae present. By substitution from equation (4) this gives:

$$\frac{\bar{y}}{x_0} = \frac{1-C^b}{1-C}. \quad (6)$$

Regarding the synthetic processes of algal growth to be irreversible, equations (4) and (6) give the respective specific radioactivities of  $\text{CO}_2$  and the total algal carbon as a function of the fractional quantity of  $\text{CO}_2$  remaining in the system.

If the conversions were totally reversible, the spe-

<sup>4</sup> Error estimates not specified.

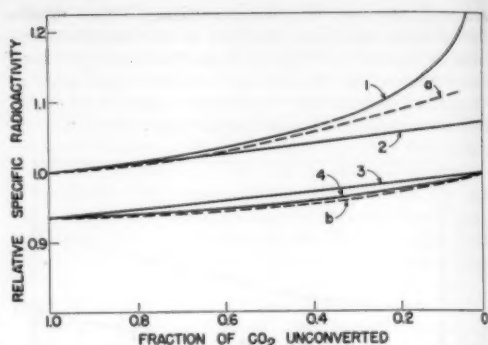


FIG. 2. Change in theoretical specific radioactivities of dissolved  $\text{CO}_2$ -bicarbonate and algal carbon with growth: 1,  $\text{CO}_2$ -bicarbonate during "irreversible" growth (equation 4); 2,  $\text{CO}_2$ -bicarbonate during reversible growth (equation 7); 3, algal carbon during "reversible" growth (equation 8); 4, algal carbon during "irreversible" growth (equation 6); a,  $\text{CO}_2$ -bicarbonate, experimental; b, algal carbon, experimental (see Fig. 1).

cific radioactivity of the total algal carbon should remain a constant fraction of the specific radioactivity of the remaining  $\text{CO}_2$ . When  $bx$  is substituted for  $\bar{y}$  in (5), the specific radioactivity of  $\text{CO}_2$  is defined by:

$$\frac{x}{x_0} = [b(1-C) + C]^{-1}, \quad (7)$$

and similarly:

$$\frac{\bar{y}}{x_0} = b[b(1-C) + C]^{-1}. \quad (8)$$

Use of the empirically derived value of  $b$ , 0.935, in equations (4), (6), (7), and (8), respectively, results in the 4 unbroken curves of Fig. 2 for inorganic and algal carbon predicted from the limiting conditions. The experimental data are repeated in Fig. 2 as broken curves. A comparison of the hypothetical curves with the measured specific activity of  $\text{CO}_2$  during the final half of the growth period seems to show that, under these conditions, some of the conversion is reversible and some is not. However, the difference between the  $\text{CO}_2$  in solution and the algal carbon from our theoretical curves for irreversible growth after 70 per cent conversion is only 11 per cent, as contrasted with the value of 24 per cent computed from Weigl's data (14). In 12 separate comparisons of  $\text{CO}_2$  from the gas phase with  $\text{CO}_2$  in the medium, we found the isotope level to be only  $1.58 \pm 0.03^3$  per cent lower in the gas phase, whereas Weigl found this difference to be 7 per cent. In Weigl's experiment the large isotope effect with carbon-14 was accompanied by a carbon-13 effect similar to ours. For these reasons it must be concluded that our carbon-14 results are in substantial disagreement with his.

Data such as these cannot be interpreted so as to allow a prediction of the biological isotope effect in other experiments. The need for evaluation of the isotope effect depends upon the type and goal of the research. In many investigations where carbon-14 is

used as a tracer, the effect may be neglected because small differences do not influence the interpretation of results. In other types of experiments, such as those involving retention and excretion of potentially radioactive carbon compounds, interest is primarily centered on the behavior of the isotope per se and, since isotope effects contribute to this behavior, it is unnecessary to evaluate them. In a few types of experiments, however, especially those in which kinetic tracer data are employed for a quantitative interpretation of a natural process, isotope effects should be experimentally evaluated and considered in the interpretation.

#### References

1. BIGEISEN, J. *Science*, **110**, 14 (1949).
2. ———, *J. Chem. Phys.*, **17**, 675 (1949).
3. UREY, H. C. *J. Chem. Soc.*, 562 (1947).
4. BREUSCH, F., and HOFER, E. *Klin. Wochschr.*, **13**, 1815 (1934).
5. STEWART, W. W., and HOLCOMB, R. *J. Am. Chem. Soc.*, **56**, 1422 (1934).
6. UREY, H. C. *Science*, **108**, 489 (1948).
7. SCHOENHEIMER, R., and RITTENBERG, D. *J. Biol. Chem.*, **127**, 285 (1939).
8. LASNITZKI, A., and BREWER, A. K. *Nature*, **142**, 538 (1938).
9. ANDERSON, E. C., and LIBBY, W. F. *Phys. Rev.*, **51**, 64 (1951).
10. LIBBY, W. F., ANDERSON, E. C., and ARNOLD, J. R. *Science*, **109**, 227 (1949).
11. MURPHY, B. F., and NIER, A. O. *Phys. Rev.*, **59**, 771 (1941).
12. NIER, A. O., and GULBRANSEN, E. A. *J. Am. Chem. Soc.*, **61**, 697 (1939).
13. WEIGL, J. W., and CALVIN, M. *J. Chem. Phys.*, **17**, 210 (1949).
14. WEIGL, J. W. *The Relation of Photosynthesis to Respiration*, UCRL-590. (April 1950). Berkeley: Univ. of Calif. Radiation Laboratory.
15. BIGEISEN, J., and ALLEN, T. L. *J. Chem. Phys.*, **19**, 760 (1951).
16. KULP, J. L., FEELY, H. W., and TRYON, L. E. *Science*, **114**, 565 (1951).
17. BUCHANAN, D. L., and NAKAO, A. *J. Am. Chem. Soc.*, **74**, 2389 (1952).
18. MCKINNEY, C. R., et al. *Rev. Sci. Instr.*, **31**, 724 (1950).
19. SWICK, R. W., BUCHANAN, D. L., and NAKAO, A. *Anal. Chem.* (in press).
20. STEVENS, W. H., PEPPER, J. M., and LOUNSBURY, M. J. *Chem. Phys.*, **20**, 192 (1952).
21. RAYLEIGH, LORD. *Phil. Mag.*, **4**, 321 (1902).

## Forest Ray Moulton: 1872-1952

A. J. Carlson

Department of Physiology, University of Chicago, Chicago, Illinois

DR. F. R. MOULTON was born in 1872 on a farm in the clearing forest of southern Michigan, now the village of LeRoy. He was the first child in the family, followed by four brothers, all five brothers being recorded in *Who's Who in America*. F. R. Moulton graduated from Albion College in 1894. He entered the University of Chicago for his graduate training, and received his Ph.D. in astronomy and mathematics in that University in 1899. But three years earlier (1896) he was appointed an Assistant in that University, and he continued to serve for 30 years on that faculty, from Assistant to full Professor, until he resigned in 1926. Dr. Moulton was an outstanding teacher, both to undergraduates and graduate students.

Even sixty years ago there were a number of outstanding scientists on the faculty of the University of Chicago, none greater than the geologist T. C. Chamberlin. At the turn of the century Dr. Moulton collaborated with Dr. Chamberlin in developing the challenging *Planetesimal Hypothesis* of solar system evolution.

In 1923 sixteen members of the faculties in the natural sciences (including psychology) collaborated in planning and giving a six-month elective course for college freshmen, called *The Nature of the World and of Man*. Dr. Moulton, as one of the sixteen, presented astronomy. After two years experience with this new

type of college course, each of the sixteen faculty participants agreed to prepare a chapter on his specific subject for a book. When the sixteen drafts were ready, all the participants spent one evening a week for four months in commenting and criticizing each chapter. Dr. Moulton proved himself a very competent critic, revealing his basic understanding of all phases of the natural sciences, even though his own specialty was mathematical astronomy. This may be called a *freshman experience*, very useful for Dr. Moulton's later significant service as Secretary of our Association. In 1937 Dr. Moulton became the editor of the revised edition of the book, *The Nature of the World and of Man*, now issued under the title, *The World and Man as Science Sees Them*.

When Dr. Moulton resigned from the University of Chicago faculty in 1926, he became a business man, as the financial director of the Utilities Power and Light Corporation of Chicago. In 1932 he became the Director of Concessions of the Chicago World's Fair. The concessions were the main source of income to meet the great expenses of the Fair, and the financial depression added to Dr. Moulton's burdens. He came out financially a victor, but with a serious coronary heart injury, from which he made a very good recovery. Evidently there was more to that man, F. R. Moulton, than a superior cerebrum.

When Dr. Moulton became the permanent secretary



of the AAAS in 1937, the journals, *SCIENCE* and the *SCIENTIFIC MONTHLY*, were still the property of Dr. J. McKeen Cattell. When the Association took over these journals, the editorial and financial burdens were great. The AAAS had no office space of its own in Washington in 1937; it was the guest of the Smithsonian Institution. The acquisition of the present quarters on Massachusetts Avenue called for careful planning and much work to raise funds. In 1937, the direct membership of the Association was less than 20,000. When Dr. Moulton retired from the Washington office in 1946, the direct membership had reached 43,000, largely the result of his plans and efforts. The scientific conferences, symposia, and publications (20 volumes) were other burdens on Dr. Moulton's active mind, and so were the efforts to make the Annual Meeting of the Association a more and more significant contribution to the advancement of science and human welfare.

Dr. Moulton's former students in the University of

Chicago agree that he was a great teacher. This is also indicated in the last sentence of his chapter on astronomy, written for college freshmen in 1926: "The orderliness of the universe is the supreme discovery in science; it is that which gives us hope that we shall be able to understand not only the exterior world but also our own bodies and our own minds." And this last sentence of his 1937 chapter, directed to all adult citizens: "Though science has placed us on an eminence from which we see very far beyond our horizon there still lies a challenge unknown." His achievements, his integrity, and his industry were recognized by honorary degrees awarded him by several colleges; by early (1910) election to membership in the National Academy of Sciences and as a Fellow of the Royal Astronomical Society of Great Britain.

Colleagues who knew Dr. Moulton most intimately over many of his 80 years will agree that he was a man of outstanding ability, integrity, and industry; that he devoted his life to science and human welfare.



## News and Notes

### National Entomological Societies Merge

ON January 1, 1953, the two national entomological societies, the former Entomological Society of America and the American Association of Economic Entomologists, were united. The resultant single society, representing all phases of the science of entomology, has taken the appropriate name, The Entomological Society of America. Each of the amalgamating societies brings a distinguished reputation into the new organization.

With entomologists of the state agricultural experiment stations taking the initiative, the American Association of Economic Entomologists was organized at a meeting in Toronto, Canada, in 1889. The Entomological Society of America was organized in 1906 in response to a need for a society in which emphasis would be placed on non-economic phases of entomology. Professor J. H. Comstock, of Cornell University, himself a charter member of the Association, was chosen as the first president of the Entomological Society; and the two societies have held what were to all intents and purposes joint meetings every year, with the exception of 1942 when conditions resulting from the war caused omission of the annual meetings, and 1945 when the Association met at Dallas, Texas, and the Entomological Society met with the American Association for the Advancement of Science at St. Louis, Missouri.

Although the two organizations had supposedly different interests, it was apparent from the beginning that in reality these interests overlapped widely; and as time passed, it became increasingly evident that no

clear line of demarcation between the two fields could be recognized. Merger of the two societies was considered many years earlier, but it was not until 1949 that a serious effort in that direction was undertaken. That year each society appointed a committee of three men to consider the feasibility of such a merger and to make recommendations to their respective societies. After presenting preliminary reports in favor of amalgamation at the Tampa, Florida, meetings in December, 1949, the two committees were continued as a joint committee to draw up a proposed constitution and by-laws, which were presented to the membership of each society at Denver, Colorado, in December, 1950, together with recommendations for procedure to effect the amalgamation. After due deliberation the merger proposal was submitted, by mail, to the membership of each society early in 1951. Ratification required a favorable vote by a two-thirds majority of the membership of each organization. The Association, in 1951, and the Society, in 1952, voted to ratify the new constitution, making it possible for the newly constituted Entomological Society of America to begin functioning as of January 1, 1953.

During the current year an Interim Governing Board, composed of the Executive Committees of the two former organizations, is conducting the affairs of the Society, and a full-time Executive Secretary will be selected by the Board. Dr. Charles E. Palm, of Cornell University, is President of the Society for 1953, and Herbert H. Ross, of the Illinois State Natural History Survey, is President-Elect. The Constitution provides that the President-Elect shall serve in that capacity for one year and is then to become Presi-



dent. In 1953 a President-Elect, for 1954, and the Governing Board will be chosen by members of the various Branches and Sections, in accordance with prescribed procedures.

The Society will continue to publish the *Annals of the Entomological Society of America* and the *Journal of Economic Entomology*. Each of these journals will appear in its forty-sixth volume in 1953. The Index of American Economic Entomology and Memoirs of the Thomas Say Foundations will continue to be published intermittently. *Entoma*, a directory of insect and plant disease control, will be continued, and a new monthly Bulletin, to contain current items of general interest, will also be issued.

The amalgamation should result in a society even stronger than the sum of its component parts, a society that can give effective support not only to all aspects of applied entomology but also to research in such fundamental fields as insect physiology, taxonomy, toxicology, bionomics and insecticidal chemistry. It will serve the interests of all entomological fields with a greater singleness of purpose and will enhance the already considerable reputation of the science.

C. F. W. MUESEBECK

Bureau of Entomology and Plant Quarantine  
U. S. Department of Agriculture  
Washington, D. C.

## Scientists in the News

Ernst A. Bessey, professor of botany at Michigan State College, is visiting professor in the Department of Plant Pathology at Cornell University during May. Dr. Bessey will lecture on the phylogeny of the fungi, and on European botanists and early U. S. Department of Agriculture plant pathologists, and participate in mycology field trips and conferences.

Bernard J. Brent has joined Reed & Carnrick as director of research. Dr. Brent formerly was director of the Endocrine Division of Warner-Hudnut, Inc., and vice president of Organon, Inc. For the past seven years, he has been honorary professor of endocrinology in the Department of Pharmacology at Rutgers University.

James E. Canright has been awarded a one year grant from the Penrose Fund of the American Philosophical Society for his research project entitled "The Phylogenetic Significance of the Floral Morphology and Seedling Anatomy of the Annonaceae."

Marvin Carmack, Professor of Chemistry at the University of Pennsylvania, has been appointed Professor of Chemistry at Indiana University, effective July 1.

Yun Ti Chen, who has been doing postgraduate work on the stability of co-ordination compounds at Northwestern University, has joined the research staff of the National Aluminate Corporation.

E. J. Crane, editor for the past 39 years of *Chemical Abstracts*, has won the Austin M. Patterson Award of the American Chemical Society's Dayton Section. In addition to his leadership in chemical literature, Dr. Crane has had a prominent part in the development of chemical terminology as chairman of the ACS's Committee on Nomenclature, Spelling and Pronunciation.

Lewis W. Douglas, former Ambassador to Britain, was named chairman of a mid-century conference on the evaluation and long-range use of the country's natural resources, to be held in the late fall. Vice-chairmen will be Karl T. Compton of MIT, Lewis W. Jones of Rutgers, and Herman W. Steinkraus of the Bridgeport Brass Company.

Pol Duwez, Professor of Mechanical Engineering at the California Institute of Technology, has left on a five-week mission in western Europe for the U. S. Air Force. Dr. Duwez will help set up a program under which the Office of Air Research and Development Command will sponsor basic research by western European scientists. He will visit Belgium, France, Italy, Holland, and England to inspect scientific and technical institutions which will participate.

Abraham S. Friedman, formerly research associate at the Cryogenics Laboratory of Ohio State University and Fulbright fellow at the University of Amsterdam, has joined the Thermodynamics Section of the National Bureau of Standards. Dr. Friedman will head a group of scientists investigating the properties of various deuterium compounds.

A. R. Gordon will become dean of the School of Graduate Studies at the University of Toronto on July 1. Dr. Gordon will succeed the late Harold Innis, and will continue as director of the Chemistry Department.

J. A. Gray has been honored by a special issue of the *Canadian Journal of Physics* (Feb. 1953), dedicated by his friends and former students as a tribute on the occasion of his retirement as Chown Research Professor of Physics, Queen's University, Kingston, Ontario.

Beno Gutenberg, Director of the Seismological Laboratory, California Institute of Technology, has been named recipient of the William Bowie Medal by the American Geophysical Union.

Chester I. Hall has retired after 34 years with General Electric. Mr. Hall has been the inventor of 139 patented devices, all of them of importance to everyday living. His contributions have been in the fields of refrigerator controls, synchronous time systems, aircraft and marine engines, dairy equipment, and motor starters.

Peter Hosler and Frederick W. Kavanagh have joined Eli Lilly and Company as biochemists in the Antibiotics Manufacturing and Development Division.

**Roland T. Lakey** will retire at the end of the present semester as dean of the Wayne University College of Pharmacy.

**Aaron A. Levin** has been appointed director of research and development for the Zenith Optical Company Division of Polan Industries Incorporated of Huntington, W. Va. Dr. Levin was formerly with the Scientific Bureau of Bausch and Lomb.

**Herman Mark**, head of the Division of Polymer Chemistry, Polytechnic Institute of Brooklyn, will receive the 1953 Honor Scroll of the New York Chapter of the American Institute of Chemists. Dr. Mark will be honored as teacher, scientist and researcher.

**Emil Ott**, director of research for Hercules Powder Company of Wilmington, Del., has been elected president of the American Section, Société de Chimie Industrielle. Dr. Ott's presidency will succeed that of **Worth Wade** of American Viscose Corporation.

**Charles B. Spencer**, president of Spencer, White & Prentiss of New York, has been named this year's winner of the Egleston Medal, given by Columbia University's Engineering Alumni Association.

**Bjorn Vestergaard**, Danish psychiatrist, has been appointed as research scientist to the staff of a special research project at Rockland State Hospital, New York. Dr. Vestergaard's work will be concerned with the relationship of hormones to mental disease.

**Charles L. Walker**, who has been an associate in the Bacteriology Department, University of California, since 1950, has joined the technical staff of National Aluminate Corporation to do research in industrial microbiology.

## Education

The Department of Anthropology of **Auckland University College**, N. Z., has reported as follows on the 1952 research of its staff: **Ralph Piddington** and **B. G. Biggs** made survey tours of Maori communities in Northland and the Rotorua-Te Whaiti area to select possible communities for intensive study. Mr. Biggs also completed the first of his annotated translations of unpublished manuscripts of Sir George Grey, to appear in the *Journal of the Polynesian Society*, a Maori reader, and tape recordings of Maori dialects. **W. R. Geddes** completed a report on the Sadong Dayaks of Sarawak and is engaged in a study of Maori social structure in pre-European times. **Donald Marshall** surveyed research problems in Western Samoa and the Cook Islands on an expedition of the Peabody Museum of Salem. **Helene Newbrand** is continuing a phonemic analysis of Maori in the Bay of Plenty area after work at Te Kao.

Commemorating the fiftieth anniversary of plant pathology in the **University of California**, an All-University Plant Pathology Conference was held at Berkeley in April to consider problems of mutual im-

portance on the Los Angeles, Riverside, Davis, and Berkeley campuses. The occasion served to honor professor of plant pathology, emeritus, **Ralph E. Smith** who on April 1, 1903, came to the University of California as assistant professor of plant pathology. His arrival marked the establishment of the first permanent, independent department of plant pathology in an American university.

New administrative responsibilities have been assigned to staff members of the Experimental Towing Tank Laboratory of **Stevens Institute of Technology**. **Hugh W. MacDonald**, formerly executive director, has been named deputy director to **Kenneth S. M. Davidson**, who remains as director. Advanced to assistant directors to join **Allan B. Murray**, assistant director, Industrial Research and Plant Operations, were **Wilfred C. Hugli**, plans and coordination; **John B. Drisko**, research services; and **George R. Morris**, business and finance. **Alice Winzer**, formerly research attaché at the French National Research Center in Paris, was appointed to conduct mathematical studies of problems in hydrodynamics.

## Grants and Fellowships

The **Conference of State and Provincial Public Health Laboratory Directors** invites nomination of candidates for the **Kimble Methodology Research Award** to be granted at its annual meeting for either: "A fundamental contribution which serves as a baseline for development of diagnostic methods which fall within the province of the public health laboratory" or "The adaptation of a fundamental contribution to make it of use in a diagnostic laboratory." Rules and nomination blanks may be obtained from: **F. C. Lawler**, Vermont Department of Health, 2 Colchester Ave., Burlington, Vt.; or **Henry Bauer**, Minnesota Department of Health, University Campus, Minneapolis 14, Minn.

**George Washington University** will conduct a study of the effects of aureomycin, terramycin, and penicillin on cancer, when they are used in conjunction with mustard drugs and x-ray treatments. Two grants, totaling \$21,271, have been received for this work, from American Cyanamid Company and the Charles Pfizer Company.

**Hahnemann Medical College and Hospital** announce the following research grants: \$2000 from the National Drug Co. to the Department of Medicine for a study of trypsin in vascular occlusion; \$9000 from the Atomic Energy Commission to supplement a previous grant for studies on radiation burns; \$5000 from Homemakers Products Corporation to the Division of Pediatrics and Women for research on certain of their products; \$5000 from the Office of Naval Research for work on the relationship of methionine to brain metabolism; \$4185 from the Microbiological Institute; \$6600 from the National Cancer Institute; \$11,996 from the Office of Naval Research for virus research;

\$5610 from the National Institutes of Health; and \$4285 from the Ames Company for clinical research in Decholin.

A prize of \$1000, donated by the **Kappa Delta Sorority**, will be awarded annually by the American Academy of Orthopaedic Surgeons for the best research related to orthopaedic surgery and performed by an American citizen in the U. S. Selection will be made from publications after Jan. 1, 1951, or research presented to the Academy's Committee on Scientific Investigation before Nov. 1. Information may be obtained from Dr. John J. Fahey, 1791 W. Howard St., Chicago 26.

The **National Science Foundation** has awarded to U. S. citizens 556 graduate fellowships in the natural sciences for the academic year 1953-54. The successful fellows were selected from 3298 applications from all parts of the continental U. S., Hawaii, and Puerto Rico. In addition, the foundation named 1274 applicants to an honorable mention list, which will be forwarded to the fellowship offices of U. S. graduate schools. The list of fellowship winners includes 515 predoctoral candidates and 41 postdoctoral. All fellows were selected on the basis of ability, with awards made in cases of substantially equal ability, so as to result in a wide geographical distribution. The list includes 175 winners who were awarded fellowships for the current academic year. It is expected that the announcement concerning National Science Foundation graduate fellowships for 1954-1955 will be made about Oct. 1.

**Rockefeller Foundation** grants for the first quarter of 1953 included \$250,000 to Stanford University for experimental biology; \$125,000 to the University of Pennsylvania for research in zoology; \$500,000 to the Medical School of the American University of Beirut; \$64,900 to Cornell for studies in underdeveloped areas; and \$121,000 to the Indian Council of Medical Research. Other grants were made to foreign universities and hospitals and to individuals and institutions in the form of fellowships.

## Meetings and Elections

The **American Geophysical Union** has elected the following officers, to serve until June 30, 1956; president, James B. Macelwane, St. Louis University; vice president, Maurice Ewing, Columbia University, Palisades, N. Y.; general secretary, John P. Marble, National Research Council, Washington, D. C.

Officers of the **American Oil Chemists' Society**, to serve until May, 1954, are: president, Procter Thomson, Procter and Gamble Company, Cincinnati; vice president, Charles E. Morris, Armour and Company, Chicago; secretary, T. H. Hopper, Southern Regional Research Laboratory, New Orleans; treasurer, A. F. Kapecki, Wurster and Sanger, Inc., Chicago. Members-at-large, to serve on the governing board, include H. C. Black, J. C. Konen, and W. A. Peterson.

The **Centre National de la Recherche Scientifique**, with the aid of the Rockefeller Foundation, will hold a symposium, entitled "Geometrie Differentielle," in Strasbourg, May 26-June 1. Invited participants will include: A. Weil and S. S. Chern, University of Chicago; J. L. Koszul and C. Ershmann, University of Strasbourg; L. Schwartz, University of Paris; G. Reeb, Institut Fourier, Grenoble; René Thom, Montbéliard; P. Libermann, Institut de Mathématiques, Strasbourg; A. Lichnerowicz, Collège de France, Paris; N. H. Kuiper, Landbouwhogeschool, Holland; E. T. Davies, University of Southampton; Beno Eckmann, Ecole Polytechnique Fédérale, Zurich; Dr. Dedeker, Université Libre de Bruxelles; Erhard Heinz, Institut de Mathématiques, Göttingen; and Dr. Willmore, University of Durham.

The **General Electric Company** and the Eastern New York Section of the **American Chemical Society** will be hosts for the 1953 joint summer symposium of the Division of Physical and Inorganic Chemistry of the American Chemical Society and the Solid State Division of the American Physical Society. The symposium will be held June 16-18 at the Research Laboratory of the General Electric Company, Schenectady.

The **National Academy of Sciences** has elected George W. Corner, Carnegie Institution of Washington, Baltimore, as vice president. Edwin B. Wilson, Harvard School of Public Health, Boston, and Hugh L. Dryden, National Advisory Committee for Aeronautics, Washington, D. C., were elected to the Council of the Academy. Newly elected members of the Academy include: L. V. Ahlfors, Harvard; Percival Bailey, University of Illinois School of Medicine, Chicago; H. A. Barker, University of California, Berkeley; V. H. Benioff, California Institute of Technology; J. H. Bodine, University of Iowa; Leon Brillouin, IBM; M. J. Burger, MIT; H. E. Carter, University of Illinois; D. M. Dennison, University of Michigan; J. P. Den Hartog, MIT; J. W. M. Dumond, Cal Tech; Carl Eckart, University of California, San Diego; R. Emerson, University of Illinois; J. F. Enders, Children's Hospital, Boston; P. J. Flory, Cornell; George Gamow, George Washington University, Washington, D. C.; Viktor Hamburger, Washington University, St. Louis; C. E. Hille, Yale; J. O. Hirschfelder, University of Wisconsin; J. G. Horsfall, Connecticut Agricultural Experiment Station, New Haven; E. H. Land, Polaroid Corporation, Cambridge, Mass.; D. P. C. Lloyd, Rockefeller Institute for Medical Research, New York; H. W. Nissen, Yerkes Laboratories of Primate Biology, Orange Park, Fla.; David Rittenberg, Columbia; J. F. Schairer, Carnegie Institution, Washington, D. C.; Theodore Sheldovsky, Rockefeller Institute for Medical Research, New York; J. C. Street, Harvard; Max Tishler, Merck and Company; H. G. Wood, Western Reserve University; R. B. Woodward, Harvard. Foreign Associates appointed were Jan Hendrik Oort, Observatory of Leiden, and Wilder Penfield, McGill University and Montreal Neurological Institute.

Sections of the **National Association of Corrosion Engineers** have been organized at Toronto and at Hamilton-Niagara. T. R. B. Watson of Corrosion Service, Ltd., Toronto, has been named chairman of the Toronto Section, and C. F. Makepeace of Page Hersey Tubes, Ltd., Welland, Ont., chairman of the Hamilton-Niagara Section.

A conference on "The Status of Multiple Sclerosis," sponsored by the **New York Academy of Sciences** and the **National Multiple Sclerosis Society**, was held in April in New York. Organized under the direction of H. R. Wainerdi and presided over by Pearce Bailey, director of the National Institute of Neurological Diseases and Blindness of the National Institutes of Health, the conference featured 33 eminent investigators reporting on recent research into the causes and control of the disease.

Members-at-large of the **Society of the Sigma Xi** from three different Illinois organizations joined forces with the installation of the Galesburg-Knox-Monmouth RESA Club at the Galesburg State Research Hospital. Officers of the new club are: president, Shannon C. Allen, State Research Hospital; secretary, Garrett W. Thiessen, Monmouth College; and treasurer, Curtis B. Coleman, Knox College.

The First Western Hemisphere Conference of the **World Medical Association** was held at Richmond, Va., in April, with representatives of 20 American countries in attendance. Delegates included heads of national medical associations, ministers of public health, medical college presidents and editors of national medical journals. Twenty special guest participants, covering the range of medical practice in the United States and reporting on subjects ranging from surgery of the heart and blood vessels to the use of atomic particles in cancer, took part in a series of panel discussions.

## Miscellaneous

Establishment by the **American Council on Education** of a Commission on Instruction and Evaluation, with responsibility for planning and reviewing the council's activities relating to teaching and educational evaluation, has been announced. T. Raymond McConnell, Chancellor of the University of Buffalo, is chairman.

Nine electrical engineers have been elected fellows of the **American Institute of Electrical Engineers** by its Board of Directors. Those honored were: J. W. Allen, Bendix Aviation Corporation, Teterboro, N. J.; A. G. Clavier, Federal Telecommunication Laboratories, Inc., Nutley, N. J.; A. A. Nims, Newark College of Engineering; K. M. Smith, J. C. Woods, and W. W. Wishard, Commonwealth Edison Company, Chicago; M. H. Pratt, Niagara Mohawk Power Corporation, West Syracuse, N. Y.; G. B. Scheer, Kaiser Industries, Oakland, Calif.; and Demetri Trone, General Electric Company, Rio de Janeiro.

An eight months' expedition to Africa to collect mammals, birds, and anthropological specimens has been undertaken for the **American Museum of Natural History**, under the direction of Colonel and Mrs. William J. Morden. Thomas J. Larson, graduate student of the University of California, will be expedition anthropologist, and George W. McClellan will act as general assistant.

The Philosophy of Science Group of the **British Society for the History of Science** offers a prize of \$50, open to all, for the best essay of not more than 4000 words on "What is the logical and scientific status of the concept of the temporal origin and age of the universe?" Information may be secured from the Hon. Secretary of the Philosophy of Science Group, University College, Gower St., London, W. C. 1.

J. Griffiths Davies, Associate Chief of the **Commonwealth Scientific and Industrial Research Organization**, Melbourne, has stated that the recent transformation of poor scrub land in Australia to productive pasture is only a prelude to similar improvement throughout all better rainfall areas. Opportunities for development of about 340 million acres of unimproved land in Australia are being revealed by research into deficiencies of the trace elements, such as copper, zinc, molybdenum, and cobalt.

Columbia University's **Lamont Geological Observatory** has launched a two-month expedition to the Gulf of Mexico with twelve scientists and technicians aboard the schooner *Vema*. They will investigate the possibility of an underwater canyon discovered by Columbia geologists last summer. They will also check sediments from the bottom of the gulf to determine whether the bottom was ever above water.

Chemicals wanted by the **Registry of Rare Chemicals**, 35 West 33rd St., Chicago 16, Ill., include: molybdenum carbonyl; isophthalaldehyde; thiobenzoic acid; 3,4,5-trichlorobenzaldehyde; methyl  $\alpha$ -benzylacrylate; trinaphthylmethane; 3-fluorophenylalanine; 3,3-dichloropropene-1; 2-cyanobutadiene-1,3; 2,6-dimethylheptanetriol-2,4,6; 5,5'-dichloro-6,6'-dimethylindigo; 1-methyl-4-methylolglyoxaline; 1-methyl-5-cyanomethylimidazole; 2,6-dichlorodiphenylamine; camphane aldehyde; vulbocarpine; hypertensinogen; perillaldehyde; quercetagetin; and daidzin.

An internationally recruited team of medical scientists left Geneva Headquarters of the **World Health Organization** recently for a seven-week visit to Indonesia. Under the chairmanship of Arvid Wallgren, professor of pediatrics, Royal Carolina Institute, University of Stockholm, the team will work with their Indonesian counterparts in hospital and university departments. There will also be group discussions, lectures, films, and demonstrations.

**ERRATUM.** The Dr. Howard Sloan Memorial Grant was erroneously mentioned as given for the benefit of the University of Chicago Medical School (SCIENCE, 117, 446). The grant was given to The Chicago Medical School, 710 S. Wolcott Ave., Chicago.



# Technical Papers

## Effect of Nuclear Counting Procedures on Determination of the Desoxypentose Nucleic Acid Content of Rat Liver Cell Nuclei<sup>1</sup>

S. Albert, Ralph M. Johnson, and  
Renée R. Wagshal

Detroit Institute of Cancer Research, Detroit, Michigan

Values of  $5.9\text{--}14.0 \times 10^{-6}$   $\mu\text{g}$  DNA/liver cell nucleus have been reported (1-8) in young mature rats (200-300 g). Such wide variation might conceivably result from differences in the media used in isolating nuclei, in nuclear counting techniques, or in the method of determining DNA. The work of Schneider (9) and of Davidson (10) would appear to rule out the last possibility.

While attempting to devise a medium from which clean nuclei and cytoplasmic particles could be obtained simultaneously, observations were made that were originally interpreted to indicate that the composition of the medium determined the amount of DNA finally calculated to be present in nuclei. When rat liver nuclei were prepared in a modified citric acid medium, a value of  $10.4 \pm 0.59 \times 10^{-6}$   $\mu\text{g}$  DNA/nucleus (9 determinations) was obtained. When, however, nuclei were isolated from a sucrose medium, the value was  $7.7 \pm 1.2 \times 10^{-6}$  (3 determinations).

During the course of these experiments, it was observed that considerable numbers of nuclei from the final preparations made in the modified citric acid medium adhered to the walls of the glass pipettes used for transferring samples to the counting chambers. This did not occur when nuclear counts were made on initial homogenates (prior to the isolation of the purified nuclei) prepared either in citric acid or in sucrose. The adherent nuclei were firmly attached to the glass and were removed with considerable difficulty in the cleaning process. Special care in cleaning the glass pipettes prior to use did not reduce the tendency to adhere. The importance of this as a source of error becomes apparent when it is realized that adherence of large numbers of nuclei to the glass pipettes would result in abnormally low values for the numbers of nuclei present in the suspensions and in an erroneously high estimation of DNA/nucleus. This may account for some of the variations reported in the literature. The use of concentrated acid, solvents, fixatives, or silicone coating of the pipettes did not prevent the adherence of nuclei.

The possibility of evaluating this "sticking" of nuclei as a source of error by substituting a wire loop<sup>2</sup>

for the pipette was suggested by Caroline Rant, microbiologist, of this institute. Male rats of the Fisher strain, weighing 210-230 g, were anesthetized with ether, the livers perfused *in situ* with ice-cold isotonic saline, and pulped by forcing them through a plastic tissue press. They were then homogenized in 1-2 volumes of the medium (to be described), using a motor-driven metal pestle covered with rubber and fitted to a glass tube, and the homogenate diluted to 10 volumes with the medium (subsequently referred to as the initial homogenate). A 2 ml sample of this homogenate was removed with a glass pipette and diluted to 5 ml with the medium. A drop was then placed on a hemocytometer and allowed to spread under the coverslip. For each sample a minimum of 1000 nuclei was counted in 10 hemocytometer fields of 9 RBC squares each, using a phase contrast microscope. The standard error of all homogenate and final nuclear preparation (loop and glass pipette) counts made in these experiments was  $2.8 \pm 0.24\%$ . DNA was determined as previously described (11).

Nuclei were isolated, using a medium of either 0.026 M citric acid or 0.25 M sucrose. Centrifugations were carried out in an International refrigerated centrifuge, Model PR-1, and all operations were performed in a cold room at 0° C. When citric acid was used, the initial homogenate, after a portion had been removed for nuclear counts, was centrifuged for 5 min at  $690 \times g$ . The supernatant was discarded, and the residue was resuspended in 0.026 M citric acid and re-centrifuged for 3 min at  $16 \times g$ . The residue (R2) was saved, and the supernatant was recentrifuged under the same conditions. The supernatant (S3) was saved, and the residue pooled with residue R2, resuspended and centrifuged for 3 min at  $28 \times g$ . The residue was discarded, and the supernatant recentrifuged for 3 min at  $16 \times g$ . This residue was discarded, the supernatant was pooled with supernatant S2, and centrifuged for 5 min at  $690 \times g$ . The resulting supernatant was discarded, the residue resuspended and recentrifuged for 5 min at  $690 \times g$ , and the supernatant discarded. A yellow layer on top of the residue, consisting largely of cell debris, was swirled off gently; the remaining residue was considered the final nuclear preparation. It consisted of nuclei, a small amount of nuclear membranes, and occasional bits of nuclear and nonnuclear fragments. This preparation was diluted to 20 ml, and a 1 ml aliquot was used for counting; the suspension was transferred to the counting chamber with a glass pipette. When the wire loop was used in the counting, a loopful of the nuclear preparation was introduced under the coverslip of the hemocytometer and 5 RBC squares were counted. In 8 such fields a minimum of 5000 nuclei was enumerated. The DNA

<sup>2</sup> To obtain low speeds an accessory rheostat was placed in series with the standard control of the refrigerated centrifuge (12).

<sup>1</sup> Supported by grants from the National Cancer Institute, of the National Institutes of Health, USPHS; the American Cancer Society, Inc.; the Kresge Foundation; and the Michigan Cancer Foundation.

<sup>2</sup> The loop was made from a short length of wire and consisted of several decreasing spirals at one end and a short handle at the other.

TABLE 1  
DESOXYPENTOSENUCLEIC ACID CONTENT OF RAT  
LIVER CELL NUCLEI\*  
(Medium: 0.25 M Sucrose)

| Diluting medium<br>for counting            | DNA values<br>observed $10^{-6}$<br>$\mu\text{g}/\text{nucleus}$ |
|--|--|
| 0.25 M sucrose                             | 7.9  |
|  | 8.8  |
|  | 8.0  |
| 0.25 M sucrose plus<br>0.026 M citric acid | 7.6  |
|  | 8.2  |
|  | 7.9  |
| Grand Av                                   | $8.1 \pm 0.17 \text{ SE}$  |

\* Nuclear counts were made on the initial homogenates.

content of the remaining material was then determined.

When sucrose was used, the initial homogenate, after a portion had been removed for nuclear counts, was centrifuged for 10 min at  $690 \times g$ . The supernatant was discarded, and the residue resuspended and re-centrifuged under similar conditions. The residue obtained was considered to be the nuclear preparation, and its DNA content was determined. Inasmuch as it is very difficult to see and count the nuclei in the initial homogenates when diluted with 0.25 M sucrose, a mixture of 4 ml of 0.25 M sucrose and 1 ml of 0.026 M citric acid was employed. The latter made the nuclei more prominent and more readily counted, but had no effect on the DNA/nucleus (Table 1).

TABLE 2  
DESOXYPENTOSENUCLEIC ACID CONTENT OF RAT LIVER CELL NUCLEI\*  
(Medium: 0.026 M Citric Acid)

| Medium                    | No. of<br>observations | Nuclei recovered (%) |              | DNA values observed<br>( $10^{-6} \mu\text{g}/\text{nucleus}$ ) |                |
|---------------------------|------------------------|----------------------|--------------|---|----------------|
|                           |                        | Loop                 | Pipette      | Loop  | Pipette        |
| Citric acid†              | 5                      | $63 \pm 4.2$         | $41 \pm 2.3$ | $7.5 \pm 0.2$   | $11.2 \pm 0.1$ |
| Sucrose-CaCl <sub>2</sub> | 1                      | 74                   | 54           | 7.1   | 9.2            |

\* Nuclear counts were made on both initial homogenates and final nuclear preparations.

† Figures represent average values and standard errors.

When the wire loop was used in transferring nuclear material to the counting chamber, the DNA values/nucleus of preparations made from citric acid were quite close to those obtained with nuclei prepared from sucrose (Tables 1 and 2). Whether the small difference observed ( $P=0.05$ ) was due to differences in media or to other factors is not known. On the other hand, when glass pipettes were used, considerably more DNA was estimated to be present than in both the citric acid-prepared nuclei counted using a wire loop ( $P < 0.001$ ), and those obtained from sucrose ( $P < 0.001$ ) (Tables 1 and 2). Furthermore, the recoveries of nuclei in the final citric acid preparation were lower than those obtained when using the wire loop ( $P < 0.01$ ) (Table 2).

The recovery of 63% of the citric acid-prepared nuclei in the final nuclear preparation with the loop-

counting method (Table 2) was appreciably lower than the 70-90% recovered by Hogeboom *et al.* (13) using a sucrose medium and a filtration technique. In the present experiments, a higher nuclear yield could have been obtained in the citric acid technique if the yellow layer had been allowed to remain with the nuclei, but a less satisfactory preparation would have resulted.

Attempts were then made to determine whether clean and nonclumped nuclei isolated from sucrose also adhere to the walls of the glass pipettes. Experience has shown that nuclei prepared in sucrose have a tendency to clump and are therefore very difficult to count. Several procedures have been suggested to eliminate this clumping and its attendant nonnuclear contamination. The most promising are the layering technique of Wilbur and Anderson (12) and the  $\text{CaCl}_2$ -sucrose-filtration technique of Schneider and Petermann (14). However, the nuclear preparations obtained by Wilbur and Anderson, although clean, contain clumped nuclei, and the procedure of Schneider and Petermann subjects the nuclei to the stress of vacuum and passage through closely woven cloth. A procedure was used which combined features of each method. The medium consisted of 0.25 M sucrose and 0.0009 M  $\text{CaCl}_2$ , and the nuclei were isolated by layering. Nuclear counts were made on the initial homogenates using glass pipettes, and on the final nuclear preparation using both pipettes and wire loops. On microscopic examination of the pipettes used in transferring the final

preparations, nuclei were again found to adhere to the walls. The estimated DNA/nucleus and the nuclei recovered are indicated in Table 2.

These experiments demonstrate that the use of glass pipettes as part of the procedure for counting rat liver cell nuclei in final nuclear preparations employing a medium of either citric acid or sucrose is contraindicated. The use of a wire loop circumvents the counting difficulties encountered with pipettes. When pipettes were used with the citric acid medium, the observed value of DNA/nucleus was  $11.2 \pm 0.1 \times 10^{-6} \mu\text{g}$ . On the other hand, when a wire loop was used with the citric acid medium, the observed value of DNA/nucleus was  $7.5 \pm 0.2 \times 10^{-6} \mu\text{g}$ . The latter value is quite close to the value of  $8.1 \pm 0.17 \times 10^{-6} \mu\text{g}$  DNA/nucleus observed when sucrose was used as the medium, and the counts were done on the initial homogenates.



## References

1. CUNNINGHAM, L., GRIFFIN, A. C., and LUCK, J. M. *J. Gen. Physiol.*, **34**, 59 (1950).
2. DAVIDSON, J. N., and LESLIE, I. *Cancer Research*, **10**, 587 (1950).
3. DOUNCE, A. L., et al. *J. Gen. Physiol.*, **33**, 629 (1950).
4. PRICE, J. M., and LAIRD, A. K. *Cancer Research*, **10**, 650 (1950).
5. HARRISON, M. F. *Nature*, **168**, 248 (1951).
6. LEUCHTENBERGER, C., VENDRELY, R., and VENDRELY, C. *Proc. Natl. Acad. Sci. U. S.*, **37**, 33 (1951).
7. LEUCHTENBERGER, C., et al. *Exptl. Cell Research*, **3**, 240 (1952).
8. CAMPBELL, R. M., and KOSTERLITZ, H. W. *Science*, **115**, 84 (1952).
9. SCHNEIDER, W. *Cold Spring Harbor Symposia Quant. Biol.*, **12**, 169 (1947).
10. DAVIDSON, J. N., et al. *Biochem. J. (London)*, **46**, Proc. xi (1950).
11. ALBERT, S., JOHNSON, R. M., and COHAN, M. S. *Cancer Research*, **11**, 772 (1951).
12. WILBUR, K. M., and ANDERSON, N. G. *Exptl. Cell Research*, **2**, 47 (1951).
13. HOGEBROOM, G. H., SCHNEIDER, W. C., and STRIERICH, M. J. *J. Biol. Chem.*, **196**, 111 (1952).
14. SCHNEIDER, R. M., and PETERMANN, M. L. *Cancer Research*, **10**, 751 (1950).

Manuscript received September 29, 1952.

## Biosynthesis of $C^{14}$ -Specifically Labeled Cotton Cellulose<sup>1</sup>

Glenn A. Greathouse<sup>2,3</sup>

Engineering and Industrial Experiment Station,  
University of Florida, Gainesville

Cellulose is formed biologically by a process of which the chemistry is almost unknown. In 1949 the author conceived the idea of synthesizing position-labeled bacterial cellulose from sugars labeled specifically with radioactive  $C^{14}$ . It was considered that a study of radioactive labeled celluloses synthesized by the microorganism *Acetobacter xylinum*, cultured on, or supplied with,  $C^{14}$ -specifically labeled substrates, might help to elucidate the mechanism of normal cellulose biosynthesis. Accordingly, arrangements were made for collaboration with H. S. Isbell and his associates at the National Bureau of Standards and with Milton Harris and his associates at the Harris Research Laboratories. A program was formulated for a joint project, and the Atomic Energy Commission awarded the funds for the proposed basic research. Four reports or publications have resulted from the synthesis of radioactive-labeled sugars and of  $C^{14}$ -labeled bacterial cellulose, two from the former (1, 2) and two from the latter (3, 4).

With the study of specifically labeled bacterial cellulose well under way, the author planned some experiments to learn if radioactive cellulose could be

produced in the *Gossypium herbaceum* cotton boll. It is the purpose of this paper to report the success of the experiments concerned with the biosynthesis of  $C^{14}$ -specifically labeled cellulose in a cotton boll, while attached to the plant.

**Approach.** Previous determinations by the author, as well as by other workers, had indicated that the reducing-sugar content of the cotton boll is 60-70% between the time of pollination of the flower and 21 days following fertilization. Likewise, the literature (5) showed that the primary cell wall of the cotton fiber is formed and reaches its full length during the first 20 days or so following fertilization of the flower. With this information available, it was reasoned that, if D-glucose-1- $C^{14}$  were introduced into the cotton boll through the vascular system of the plant at the time of optimum sugar translocation and cellulose synthesis, it would diffuse with the other sugars and enter into the cellulose formed by the boll. The importance of introducing the labeled sugar just at the time of optimum sugar translocation cannot be overemphasized. The best results from the standpoint of glucose-1- $C^{14}$  utilization should be obtained at the time the greatest quantity of reducing sugars is being converted to cellulose in the normal boll. This, according to the best prevailing knowledge of cellulose formation, should be approximately 21 days after fertilization of the flower.

**Procedure and Results.** Four Stoneville 2 B cotton plants were grown from seed in 6-in. pots containing soil in a greenhouse of the U. S. Department of Agriculture at Beltsville, Md. The plants grew and flowered normally. Considerable experimentation was required to develop the best procedure for introducing glucose into the boll without hindering its development. The procedure selected was to slice the stem longitudinally just below the boll with a razor blade and place the cut portion into the sugar solution. The soil in which the plant was growing was first allowed to dry to the point that the plant lost some turgor and was then watered approximately 30 min before introduction of the glucose-1- $C^{14}$ . The sugar solution is taken into the boll within a few minutes, the rate of transfer varying with the sugar content of the boll at the time of experiment. The stem and the boll were carefully taped to prevent injury and to allow normal boll development.

One experiment was performed by introducing the glucose-1- $C^{14}$  into the boll (ovary) the same day the flower was pollinated and harvesting the developing boll on the 21st day. The cotton fibers were removed, dewaxed (6, 7), and extracted with 1% NaOH. The small quantity of primary-wall cellulose remaining was assayed for radioactivity. The radioactivity of the cellulose itself was low; the extracts taken of the cellulose and of the remaining parts of the cotton boll contained most of the radioactivity introduced as glucose-1- $C^{14}$ .

Accordingly, a second experiment was performed in which 12.5  $\mu$ c of glucose-1- $C^{14}$  were introduced

<sup>1</sup> The work with which this report is concerned was conducted under the sponsorship of the U. S. Atomic Energy Commission and the U. S. Army Office of Ordnance Research.

<sup>2</sup> The author wishes to acknowledge the assistance of Nell W. Stuart of the U. S. Department of Agriculture in developing the plant-dosing technique. Thanks are also due H. S. Isbell and associates at the National Bureau of Standards for synthesizing the necessary D-glucose-1- $C^{14}$  and for assisting with the analytical procedures.

<sup>3</sup> Present address: Prevention of Deterioration Center, National Research Council, Washington, D. C.

through the sliced stems just below the 21-day boll. The treated boll matured 30 days later. The fiber from the treated boll was separated from the seeds and extracted with ethyl alcohol in a Soxhlet extractor for 5 hr (7) to remove the waxes quantitatively (6). The extracted fiber was air-dried and then boiled in 300 ml of 1% NaOH for 1 hr, the fiber being tied with thread to a glass rod to keep it well under the surface and prevent oxidation. On removal from the alkali, the fiber was rinsed in dilute acetic acid, then in water repeatedly until rinsings no longer were acid, and the sample was dried at 50° C for 2 hr in a circulating-air oven. Yield: 406 mg of purified cellulose.

The purified cellulose was hydrolyzed by the method of Monier-Williams (8). A yield of 337 mg of unpurified sugar was recovered from the cellulose hydrolyzate. The crude sugar was made up to 5 ml with water, and a 1/100-ml portion was removed for direct determination of C<sup>14</sup> activity according to the method of Schwebel, Isbell, and Karabinos (9). This procedure indicated that 5.49  $\mu$ C<sup>14</sup> were present in the 5 ml of sugar solution. Thus, approximately 44% of the radioactive C<sup>14</sup> was converted to cellulose and recovered in the glucose molecule.

The 5 ml (less the 1/100 ml used for analysis) of sugar solution were passed through approximately 5 g of Darco G-60 and Celite according to the method of Whistler and Durso (10) and washed with water (10 times the volume of adsorbent) to recover the glucose. This procedure separates the monosaccharides from the disaccharides, the monosaccharides coming off in the water wash and the disaccharides being eluted with 20% ethanol. This separation indicated 95% of the cellulose had been hydrolyzed to glucose by the Monier-Williams method.

The water wash solution of glucose from the carbon-Celite column was freeze-dried and crystallized from methyl and isopropyl alcohol. A yield of 298 mg of crystallized glucose was recovered with a specific activity of 0.0192  $\mu$ C/mg or 3.459  $\mu$ C/mM as determined by the vibrating reed electrometer. A 75-mg sample of the radioactive glucose was oxidized by oxygen in aqueous potassium hydroxide<sup>4</sup> to remove carbon 1. The resulting potassium D-arabonate was separated and recrystallized. A 5.82-mg sample of the purified potassium D-arabonate was oxidized and the activity of the resulting CO<sub>2</sub> determined by use of a vibrating reed electrometer. The drift rate was equivalent to 1.1 dps for the 5.82 mg sample. This corresponds to  $1.04 \times 10^{-3}$   $\mu$ C/mM. Dividing  $1.04 \times 10^{-3}$  by 3.459 (activity of original glucose from cotton)  $\times 100 = 0.03\%$ . Hence 99.97% of the activity was in carbon 1 of the glucose molecule.

These data are most interesting, since they constitute strong evidence that the glucose-1-C<sup>14</sup> was synthesized to cellulose directly by an enzyme system of the cotton boll. No one has previously traced the synthesis of cellulose in the cotton boll through the in-

roduction of glucose-1-C<sup>14</sup> and thus there are no prevailing theories as to the mechanism involved in cellulose synthesis. From the above data one might theorize that the glucose is polymerized directly, possibly receiving the essential energy through phosphorylation.

Later publications will compare the data obtained on the biosynthesis of C<sup>14</sup>-specifically labeled cellulose in the cotton boll and on that of the cellulose produced by *A. xylinum*.

#### References

1. ISBELL, H. S., et al. *J. Research Natl. Bur. Standards*, **43**, 163 (1952).
2. ISBELL, H. S., and KARABINOS, J. V. *Ibid.*, 438.
3. GREATBATCH, G. A., et al. The Biosynthesis of C<sup>14</sup>-Specifically Labeled Cellulose by *Acetobacter xylinum*. Abst., 2nd Biochem. Congr., Paris (1952).
4. MINOR, F. W., et al. The Biosynthesis of C<sup>14</sup>-Labeled Cellulose by *Acetobacter xylinum*. Abst., 12th Intern. Congr. Pure Appl. Chem., N. Y. (Sept. 1951).
5. ANDERSON, D. B., and KERR, T. *Ind. Eng. Chem.*, **30**, 48 (1938).
6. MARSH, P. B., et al. *Textile Research J.*, **20**, (5), 288 (1950).
7. CONRAD, C. M. *Ind. Eng. Chem., Anal. Ed.*, **16**, 745 (1944).
8. MONIER-WILLIAMS, G. W. *J. Chem. Soc.*, **119**, Part 1, 803 (1921).
9. SCHWEBEL, A., ISBELL, H. S., and KARABINOS, J. V. *Science*, **113**, 465 (1951).
10. WHISTLER, R. L., and DURSO, D. F. *J. Am. Chem. Soc.*, **72**, 677 (1950).

Manuscript received January 16, 1953.

### A Cage for Rearing Predator-Prey Populations of Mites<sup>1</sup>

Thomas Burnett

Dominion Parasite Laboratory,  
Belleville, Ontario, Canada

At the Belleville laboratory, studies are in progress on the establishment of a predator-prey system, the grain mite *Acarus siro* L. being used as the prey species and other mites as predators. The initial phase of this work dealt with the development of a universe that would provide the following requirements for the studies: (a) the mites should develop in as natural a state as laboratory conditions would allow; (b) the populations of predator and prey should be spread in a single plane so that most of the individuals could be observed and counted; (c) it should be possible to record the distribution of the predator and prey populations; (d) physical factors such as temperature, humidity, and the surface over which the predator searches for prey should be regulated; (e) if necessary, it should be possible to add fresh food as required; and (f) it should be possible to replicate each experiment. A description of a cage designed to satisfy these conditions forms the basis of this note.

The cage consists of 2 square sheets of glass held 1/2 in. apart by strips of acrylic resin plastic on the four sides (Fig. 1). The base of the cage consists of a sheet of double-diamond glass with smoothed edges.

<sup>1</sup> Contribution No. 3013, Division of Entomology, Science Service, Department of Agriculture, Ottawa, Canada.

<sup>4</sup> An unpublished procedure of H. S. Isbell and associates of the National Bureau of Standards.

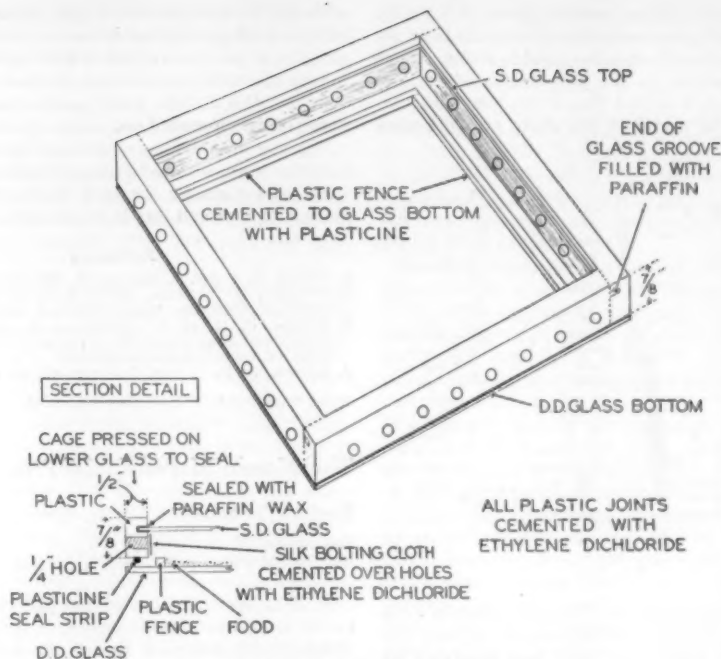


FIG. 1. Cage, with details of construction.

A plastic fence that retains the food is fastened upon the glass plate with plasticine. The sides of the cage are a unit with the glass top. In making the top of the cage the sides are cut from a sheet of plastic  $\frac{1}{2}$  in. thick (the length of the sides depends, of course, on the size of the cage), the groove to hold the glass top is cut, and the ventilating holes are drilled. Silk bolting cloth is then cemented, by means of the solvent ethylene dichloride, on the inside of the plastic strips between the groove that holds the glass top and the bottom. For low-density populations of the grain mite, 15 XX cloth is satisfactory, but for high-density populations a cloth of finer mesh is preferable. The four sides are then placed around a sheet of single-diamond glass and the corners cemented together. Finally, the glass is sealed in the groove cut in the plastic with paraffin wax. The top and bottom parts of the cage are sealed by a ribbon of plasticine. The ribbon is formed by forcing black plasticine through a small circular opening in the end of a compression tube similar in design to a hand-operated grease gun.

In practice, a thin layer of food is sifted upon the bottom of the cage and removed from the area outside the plastic fence. Foods that tend to become moldy at high humidities are sprayed with a 2% solution of Shirilan N.A. in 50-70% ethyl alcohol. After the food has completely dried, the top of the cage is sealed in place. Each time individual predators or

prey are added or removed from the cage it is necessary to break the plasticine seal. Afterward a fresh ribbon of plasticine is applied. For the observation and census of the mites a binocular dissecting microscope is suspended above the cage and a light source is placed underneath the cage. Movement of the mites can be prevented if necessary either through the use of carbon dioxide or by cooling the cage.

Populations of the flour mite generally move to the edge and corners of the food area when they are introduced into the cage. It is not known whether this indicates a lack of uniformity within the cage or whether it is characteristic of the species. The distribution becomes more uniform a few months later, when the population has increased or has been subject to attack by the predator.

Manuscript received September 22, 1952.

## Growth and Regeneration in *Hevea* Seedlings

T. J. Muzik

Federal Experiment Station, U. S. Department of Agriculture, Mayaguez, Puerto Rico

In view of the present interest in Para rubber, *Hevea brasiliensis* (Ex. ADR. Juss.) Muell. Arg., methods of vegetative propagation are of considerable importance. Twigs of mature trees do not form roots, whereas stem

cuttings from the base of seedling plants root readily (1). Clonal multiplication is usually done by budding, but the effect of stock on scion yield is still unsettled (2). A new method for the propagation of cuttings from seedlings is described here in the hope that such material may be useful in the study of stock-scion relationships.

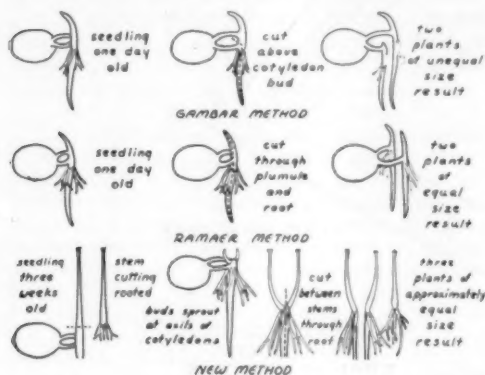


FIG. 1.

Coster (3) reports the use of twin seedlings in Java for stock-scion experiments. Methods of splitting the seedlings of *H. brasiliensis* have been described by Loomis (4) and Dykman (2). The two best-known methods are the Ramaer, in which germinated seedlings about 3 days old are split into two equal parts by a vertical cut passing through the plumule and taproot between the cotyledon petioles; and the Gambar, in which the vertical cut does not completely divide the main stem but begins at a point slightly above the axil formed by one of the cotyledon petioles with the main stem and passes obliquely inward to the center of the main stem and downward between the cotyledon petioles, dividing the taproot into two equal parts. Both these methods produce "twin" plants (Fig. 1).

In studies of regeneration in *H. brasiliensis* a new method of splitting *Hevea* seedlings to obtain three or more plants was developed.<sup>1</sup> The young stem is cut off at 6 weeks of age and planted as a cutting. As previously shown (1), this is the age at which the seedling separates from the seed and rooting potential is highest. Removal of the stem stimulates the growth of buds at the base, in the axils of the cotyledons. Generally one sprouts in each axil. When these have reached a height of about 6 in., the taproot can be split, and thus three plants are obtained (Fig. 1). The shoots from the axils of the cotyledons can also be rooted, although they take about 6 days longer than the primary stems. Removal of these shoots induces new buds to grow, and these may be removed and planted, thus offering a potentially unlimited source of material.

<sup>1</sup> From a dissertation submitted, as a partial requirement for the Ph.D. degree, to the Graduate School, University of Michigan, Ann Arbor.

although the sprouts tend to get smaller each time a cutting is taken. Under greenhouse conditions, as many as 7 plants have been obtained from one seed by maintaining the cuttings in a damp chamber. It seems possible that this division could go on almost indefinitely under optimum conditions.

The new method is advantageous because more than two plants are obtained from each seed and less mortality occurs among the split plants, since a better balance of root and leaf is maintained.

#### References

1. MUZIK, T. J. *Am. J. Botany*, **34**, 587 (1947).
2. DYKMAN, M. J. *Hevea, Thirty Years of Research in the Far East*. Waltham, Mass.: Chronica Botanica (1951).
3. COSTER, C. H. In P. Honig and F. Verdoorn (Eds.), *Science and Scientists in the Netherlands Indies*. Waltham, Mass.: Chronica Botanica (1945).
4. LOOMIS, H. F., *J. Agr. Research*, **65**, 97 (1942).

Manuscript received September 17, 1952.

## The Nature of Perceptual Processes

Rayford T. Saucer

Department of Psychology,  
Georgia Institute of Technology, Atlanta

In 1912 Wertheimer used the phenomenon of apparent motion to provide a point of entry into a new phase of psychological theory. Though other phenomena could have been used as a basis for the same theories, apparent motion is one of the most striking examples of the fact that the organism can and often does respond in relation to a perceptual process which displays attributes having no counterpart in the external stimulus complex. This process is produced by organizational forces within the nervous system of the organism.

A frequent objection to Gestalt theory is that there have been few efforts to relate the field forces postulated as an explanation of phenomena to the actual physiological mechanisms. While the concept of psychoneural isomorphism can hardly be denied a place in the theoretical structures of psychology, it is too often merely a convenience rather than an explanation. Other workers, not always professed followers of the Gestalt school, have chosen a path which may prove fruitful. The work of Lashley and Hebb in the field of neurophysiological theory is important to a synthesis of psychology in which phenomenology and psychology become parts of a rational science, a science which relies upon neither blind atomism nor metaphysical explanation. In spite of some objections, however, it is felt that field theory can be related to physiological mechanisms by recourse to study of the phenomena that provided the initial impetus to the move away from mechanical linkage of the stimulus to the perception or to the response by circumventing the organism.

By applying electronic control to the switching of light sources, investigations into the problem of ap-



parent motion have been facilitated at the Psychological Laboratories of the University of Florida. The problem has been divided into four areas; (1) parameters, (2) the relationship between real and apparent motion, (3) the relation between motion and other organized processes, and (4) the existence of some features of the organization which cannot be related to the direct sensory input. It will be noted that none of these can be said to represent any radical departure from the essential nature of the problem as outlined by Wertheimer (1), Neff (2), and Graham (3).

A report on some phases of the first question has been prepared (4). Electronic switching techniques similar to those used by Tanner (5) have been used to control the firing of crater lamps (Sylvania R1130). By use of suitable optical techniques the light output was focused into a spot subtending  $1^\circ$  visual angle at a distance of 0.5 m from the subject. The stimulus complex consists of two such lights, 5 cm center to center, fired  $180^\circ$  out of phase and presented on a ground glass screen. The time interval  $p$  was eliminated. (Actual delay between presentation of lights approaches  $5 \mu$  sec.) Flash rates of 4 cps/light were sufficient to give a perception of Beta movement just slightly less than 100% of the time over a 5-min recording period without previous dark adaptation. Subjects who had been prepared with a period of 5 min or more of dark adaptation reported a periodic fluctuation of perception of Beta movement. This fluctuation has been related to the dark adaptation time. Analysis of variance supports this conclusion at better than the 1% level of confidence.

It is concluded that, although the parameters subsumed under Korte's laws are adequate in view of prior experimentation, they are not complete and should be revised. Since there is a high degree of apparent motion in the absence of any pause time  $p$ , it is concluded that in the relationship between the degree of motion and the stimulus complex some purpose might be served by substituting rate for pause time. The formalized statement is then freed from the restriction imposed by a vanishingly small value of  $p$ . In addition, some expression of the physiological state of the organism may be in order, since it has been shown that dark adaptation can be a determiner of the degree of apparent motion. It is also concluded that any statement of optimal motion must include its duration in time. The other attributes of apparent motion are such that they can be included in the expression  $M_t$ , where this expression includes apparent motion through some time interval. Except for motion, the qualities of the moving stimulus or Beta object do not differ from the qualities of the primary stimulus except under special conditions to be considered later.

Examination of the stimulus object, including both its temporal and its spatial ordering, shows that alternate areas of the screen are illuminated at a rate of 4 flashes/sec/area. The illuminated areas provide retinal stimulation which eventually results in neural proc-

esses in the occipital cortex, where they are projected on a psychospatially isomorphic projection plane. This system is to be considered isomorphic in terms of spatial attributes rather than in terms of neurospatial or neurological architecture. Thus between any two points  $x$  and  $y$  in the distant spatial organization there is an area  $xy$ . Any configuration present in  $xy$  will be so perceived, although the neurological processes responsible for the perception of the configuration need not be so located in the neurological architecture of the occipital cortex.

The isomorphic areas  $x'$  and  $y'$  will be considered primary areas, or areas in which stimulation is a direct result of the routing of the nerve impulse from the receptor. They will occupy an area which is then psychologically isomorphic to real or physical space in terms of perceived relationships between  $x$  and  $y$ . The area upon which  $x'$  and  $y'$  are projected is then viewed as a field composed of cell assemblies or matrices and other areas which are statistically uniform unless they are incorporated into other matrices by existing native or linked networks. Non-linked but prebiased cell groups which are brought to a point near conduction by summative processes may also be considered matrices. Since the term "cell assembly" refers in general to closed circuits, matrix will be used to designate both such assemblies and nonlinked groups possessing similar levels of stimulation or activity. In view of the nature of these matrices with regard to incomplete isolation of the individual cell, it is felt that any excitation will not only be confined to its proper matrix but will also excite adjacent cells in proportion to their degree of prior excitation by both prior stimuli and "noise" or statistical excitation.

That color matrices are a permanent part of the field is indicated by the following experiment. If a stable spot of colored light is exposed between the two stimulus lights, the resulting phenomenon is one of apparent motion in which the moving white spot passes through the colored spot with no change in quality or planar depth. If the stationary spot is brighter, a displacement of the moving spot results, so that the moving spot appears to go behind the stationary spot. If, however, the two lights are of equal brightness and of different hue, they may excite coexistent color matrices in the same plane.

The resulting phenomenon is then one of two color films in the same plane. Observers report that the two color films occupy the same space and are in the same plane. If the intervening spot is of such a nature that it inhibits the formation of the coexistent matrix, the process is altered, the moving spot going either behind the stationary spot or clearly in front of it. It is concluded that in such cases the process which occurs as a result of the stationary spot blocks out the intervening area so thoroughly that the motion must re-route itself in another plane.

If the two lights used to produce Beta motion are viewed through monocular filters of different hue, color mixing takes place. This is believed to indicate that,

whereas color perception is a function of both the discrete retinal cell excited and its cortical ending, color mixing need not take place in the cortex. That this statement implies a much wider range of color receptors than is postulated by the Young-Helmholtz theory is not denied.

The cortical projection plane is viewed as a construct which has some psychological validity. It is isomorphic to real space and is a field composed of cell assemblies and matrices in varying degrees of excitation, but it is not a uniform field, since there is some evidence for the existence of two organized processes which are both actually isomorphic to some distant area  $xy$ . Stimulation of areas  $x'$  and  $y'$  results in an outward spreading of excitation by virtue of the mechanisms postulated earlier. In the case of apparent motion, this spreading outward may, if continued, result in the excitation of areas which are psychospatially isomorphic to the distant area  $xy$ . Under proper repetitive conditions matrices similar to those activated by the distant stimulus may participate in an organization which will be the psychological reproduction of the distant stimulus. If the distant stimuli are congruent in all qualities, the end process is one of apparent motion, since there is now an excitation for the area isomorphic to distant area  $xy$ . Hence there is a sensory-sensory transfer of an entire organized process. This statement is supported by the fact that, if the distant stimuli are covered by congruent 1-mm grids, the entire grid pattern is transferred as an integral part of the Beta motion. The Beta object differs somewhat in the degree of resolution, indicating either some nonlinearity in the transmission network or difficulty in experimental procedure.

There is apparently a correlation between the decay time of a matrix so activated and the rate of stimulation necessary to produce optimal motion. All existing calculations, including those given by Wertheimer (1), approximate the normal alpha rhythm. (It has been observed that optimal motion is better if the distance and size as well as the intensity of the stimulus are optimized with regard to a repetition rate of 4-12 serial flashes/sec.) Time constants involved also agree closely with those determined for Gamma motion.

The entire process bears a close resemblance to the problem of measurement in general, there being considerable evidence for the operation of an uncertainty principle. If the speed of the apparent motion is reduced, the complex breaks down into extreme detail (alternation) without motion. Under these conditions the distant stimulus can be described in minute detail. In the range of repetition wherein optimal motion is found, some motion as well as some detail can be measured. If the speed of motion is increased sufficiently, a new phenomenon is found to appear. This motion has been called Omega movement. It is similar to Phi, except that in general "etwas" is present. Observers report well-organized movement of "something" which is best described as a moving shadow, without detail but possessing withal considerable thing quality. It

is then operationally something in motion, not just pure motion without something moving, but with a lack of detail.

In conclusion it may be stated that the examination of the processes of apparent motion has revealed evidence for the lateral transfer of complete sensory organizations, as well as some experimental evidence for the existence of processes which have been called co-existent. If it is possible to transfer an organization which is dependent upon direct sensory input, there is little reason to doubt the existence of the simultaneous transfer of other processes both sensory-sensory and sensory-association. If a concept can exist as an encoded matrix and can be so transferred, we can lay the foundation for a psychological theory of relationships and discrimination based on a vector-sum theory of the comparison of two perceptual organizations by the fusion resulting from the encoding of two such organizations in coexistent matrices where the previous organization of the physiological system is of such a nature that neither matrix will be favored. It is believed that this theory may have implications with regard to both conditioning and learning theory as well as perceptual organization.

#### References

1. WERTHEIMER, M. *Z. Psychol.*, **61**, 161 (1912).
2. NEFF, W. S. *Am. J. Psychol.*, **48**, 1 (1936).
3. GRAHAM, C. H. In S. S. Stevens, Ed., *Handbook of Experimental Psychology*. New York: Wiley (1951).
4. SAUCEB, R. T. Unpublished Master's Thesis, Univ. of Florida (1950).
5. TANNER, W. F., JR. *Science*, **112**, 201 (1950).

Manuscript received October 10, 1952.

### Inhibition of Tobacco Mosaic Virus Biosynthesis by 2-Thiopyrimidines<sup>1</sup>

Frank L. Mercer, Taylor E. Lindhorst, and Barry Commoner<sup>2</sup>

St. Louis College of Pharmacy and Allied Sciences, St. Louis, Missouri

We have previously reported that thiouracil, an analog of uracil, inhibits tobacco mosaic virus (TMV) formation in tobacco leaf tissue (1). These studies have been extended to a series of purine and pyrimidine derivatives.

Experimental material consisted of tobacco leaf discs inoculated with purified TMV and cultured in nutrient according to a method previously described (1). The TMV content of the discs at various times after inoculation was determined by the method of Commoner *et al.* (2). Results are reported as percentage of inhibition at the time at which untreated inoculated tissue contains a maximal amount of TMV (260 hr after inoculation).

<sup>1</sup>This work was supported by a research grant (E-250) from the National Institutes of Health, U. S. Public Health Service.

<sup>2</sup>Henry Shaw School of Botany, Washington University, St. Louis, Missouri.



Four uracil derivatives (5-bromouracil, 6-hydroxy-uracil, 6-methyl-2-thiouracil and 6-propyl-2-thiouracil) gave no inhibition in concentrations up to  $10^{-3}$  M. Substances which are effective inhibitors are listed in Table 1. Inhibitory effectiveness appears to be a com-

TABLE 1

PYRIMIDINE AND PURINE DERIVATIVES THAT INHIBIT TOBACCO MOSAIC VIRUS SYNTHESIS

| Compound           | Concentration (M) | Percentage inhibition |
|--------------------|-------------------|-----------------------|
| 2-Thiouracil       | $10^{-4}$         | 90-100                |
| 2-Thiocytosine     | $10^{-4}$         | 90-100                |
| 2-Thiothymine      | $10^{-3}$         | 90-100                |
| 2,6-Diamino purine | $10^{-4}$         | 80                    |
| 8-Azaguanine       | $10^{-5}$         | 60                    |

mon characteristic of the 2-thiopyrimidines, although 2-thiothymine is somewhat less active than the uracil and cytosine derivatives. Two purine analogs, 2,6-diaminopurine and 8-azaguanine (5-amino-7-hydroxy-1H-v-triazolo [d] pyrimidine), which have been found to inhibit virus multiplication (3-5), are less effective than the thiopyrimidines. The results with 8-azaguanine confirm the observations of Mathews (5), who showed that this substance reduces the number of local lesions formed by lucerne mosaic virus-inoculated leaves of *N. glutinosa*.

In order to ascertain the point of attack of the thiopyrimidines, attempts were made to reverse their inhibitory effect by adding uracil, cytosine, and thymine to the nutrient medium. It was found that of these natural nitrogen bases, only uracil is capable of suppressing the inhibition that is due to the thiopyrimidines (Table 2). This result suggests that the inhibitory effect of thiocytosine and thiothymine is not due to interference with cytosine and thymine metabolism. On the contrary, the thiopyrimidines appear to have a common point of attack, a process which requires uracil.

These data confirm the earlier conclusion (1) that

TABLE 2

EFFECT OF PYRIMIDINES AND THIOPYRIMIDINES ON THE BIOSYNTHESIS OF TOBACCO MOSAIC VIRUS IN TOBACCO LEAF TISSUE (Virus Present—Percentage of Control)

| Concentration (M) pyrimidine | Thio-uracil $10^{-4}$ M | Thio-cytosine $10^{-4}$ M | Thio-thymine $10^{-4}$ M |
|------------------------------|-------------------------|---------------------------|--------------------------|
| None                         | 7                       | 10                        | 5                        |
| $10^{-4}$ Uracil             | 15                      | 82                        | 37                       |
| $10^{-5}$ "                  | 88                      | 140                       | 103                      |
| $10^{-3}$ "                  | 88                      | 90                        | 60                       |
| $10^{-4}$ Cytosine           | 3                       | 10                        | 4                        |
| $10^{-5}$ "                  | 5                       | 12                        | 5                        |
| $10^{-3}$ "                  | —                       | 15                        | 10                       |
| $10^{-4}$ Thymine            | 3                       | 18                        | 3                        |
| $10^{-5}$ "                  | 3                       | 28                        | 4                        |
| $10^{-3}$ "                  | —                       | 33                        | 13                       |

TMV-synthesis is uracil-dependent. The observation that 2,6-diaminopurine inhibits TMV synthesis is in agreement with a recent report by Ryzhkov and Marchenko (6). These authors also find that thiouracil is an effective inhibitor and is reversed by uracil.

# References

1. COMMONER, B., and MERCER, F. L. *Arch. Biochem. Biophys.*, **35**, 278 (1952).
2. COMMONER, B., et al. *Arch. Biochem.*, **37**, 271 (1950).
3. MOORE, A. E., and FRIEND, C. *Federation Proc.*, **10**, 385 (1951).
4. THOMPSON, R. L., et al. *J. Immunol.*, **65**, 529 (1950).
5. MATHEWS, R. E. F. *Nature*, **167**, 892 (1951).
6. RYZHKOV, V. L., and MARCHENKO, H. K. *Doklady Akad. Nauk, U.S.S.R., Leningrad*, **96**, 637 (1952).

Manuscript received September 2, 1952.

## A Snail Intermediate Host of the Rabbit Parasite *Hasstilesia tricolor* (Trematoda: Brachylaemidae)

William B. Rowan

Department of Entomology, Cornell University, Ithaca, New York

*Hasstilesia tricolor* (Stiles and Hassal, 1894) is a small fluke frequently found in the small intestine of certain species of rabbits of the genera *Sylvilagus* and *Lepus*. Around Ithaca, an infected rabbit may contain as many as 15,000 flukes, and reports of 30,000-40,000 occur in the literature (1). The occurrence of such heavy infections in wild rabbits has led to considerable speculation on the life cycle of this fluke. Several workers have attempted to determine the mode of transmission, and some have suggested that a direct life cycle may exist in this species (2). Flannery (3) attempted to feed eggs from *H. tricolor* to laboratory rabbits, but was unable to demonstrate direct development. Hendrickson (4) and Haugen (5) both found the fluke more prevalent in rabbits taken from low, marshy ground, and the latter author indicated that the most severe infections occurred in winter. Harkema (6), working in an area which showed a "paucity of fresh water," could find no correlation between type of habitat or season and the number of flukes per rabbit.

The writer has recently found that in the vicinity of Ithaca a small land snail belonging to the genus *Vertigo* serves as the intermediate host of *H. tricolor*. Henry van der Schalie compared the snail with specimens in the University of Michigan Museum of Zoology and provisionally identified it as *Vertigo ventricosa* form *elatio* Sterki. A series of these snails has been placed in the parasitological collection of the Department of Entomology at Cornell University. Of 47 snails collected in one survey area during the summer of 1952, 31 were found to be infected with motile branched sporocysts in various stages of development. Mature sporocysts contained hundreds of spinous,

unencysted brachylaemid metacercariae measuring  $48 \mu \times 34 \mu$ .

During the latter part of the summer a domestic rabbit was caged on the ground in a field of orchard grass and goldenrod where infected *V. ventricosa* form *elator* were known to occur. This rabbit was returned to the rabbitry after 2 weeks; it began to pass eggs of *H. tricolor* 25 days after exposure. A second rabbit was maintained in the rabbitry and fed infected snails. Ten days after the last feeding of snails this rabbit was examined and found to contain a number of partially grown flukes measuring about  $250 \mu$  in length. This latter experiment was repeated, using two test and two control rabbits. These rabbits were maintained on a diet of prepared pellets, heat sterilized hay, and water. The test rabbits were fed infected snails for 4 consecutive days. Ten days after the last feeding the two test rabbits were found to be infected with numerous partially grown specimens of *H. tricolor*. The two control rabbits were negative.

The ecology of *V. ventricosa* form *elator* has not been extensively studied. The snail is apparently capable of surviving in relatively arid habitats. In wet weather, at temperatures above  $40^\circ \text{F}$ , it becomes active and climbs to a height of a foot or more on vegetation. Since the snail is about  $1/16$  in. long it could readily be accidentally ingested by a feeding rabbit.

Further studies on the life cycle of *H. tricolor* are in progress and will be reported later.

#### References

1. N. Y. State Conservation Dept. 25th Ann. Rept. (1935).
2. Ibid., 26th Ann. Rept. (1936).
3. FLANNERY, W. Thesis. Ohio State Univ. (1936).
4. HENDRICKSON, G. Trans. North Am. Wildlife Conf., 2, 549 (1937).
5. HAUGEN, A. Am. Midland Naturalist, 28, 204 (1943).
6. HARKEMA, R. Ecol. Monographs, 6, 151 (1936).

Manuscript received September 20, 1952.

## Polarograms of Oxygen in Lake Water

K. G. Wood

Franz Theodore Stone Institute of Hydrobiology,  
The Ohio State University, Put-in-Bay

The dropping mercury electrode was used to determine oxygen in lake water by Manning (1). The writer was introduced to the method by W. A. Spoor, of the University of Cincinnati and of this institute, with a view to determining the oxygen uptake of small aquatic insects. Although this objective was not attained, several aspects of the application were investigated.

Polarograms of oxygen at four levels of oxygen concentration are presented in Fig. 1, for Lake Erie water (90 ppm total alkalinity). The voltage-current relationship consists of two waves, one in the region up to about 0.6 v, and the second from about 0.8 to 2.0 v. These waves are indistinguishable at 1.4 ppm of oxygen because of the slight current flow. Above

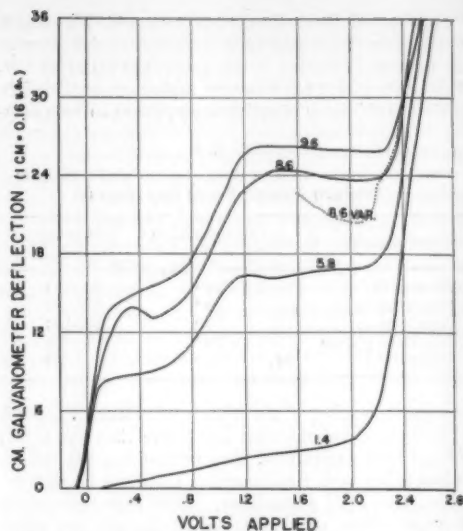


FIG. 1. Polarograms of Lake Erie water at 1.4, 5.8, 8.6, and 9.6 ppm concentrations of dissolved oxygen.

2.1 v the relationship followed was essentially that of Ohm's law at all levels of oxygen. At zero potential applied, a negative current is generally expected, as indicated by the extrapolation of the 1.4 curve. However, the calomel half-cell and external anode system was such that it acquired the polarity of the applied emf and could be made to register in either direction. The three upper curves had a basis of  $-0.16 \text{ v}$  vs. the saturated calomel electrode.

The existence of these oxygen reduction waves is well known. The points of interest here are (1) they were obtained with unmodified lake water in a flowing system, and (2) they indicate the best voltage at which to determine oxygen in such a system.

The flowing system was a plastic block drilled and fitted with 5 mm OD glass tubing. At right angles to this water line, holes were drilled in the plastic to receive the two electrodes: the cathode of marine barometer tubing; and the anode, a glass tube with a 5 mm diameter tip of Corning fine porosity, sintered glass filter. Mercury flowed through the capillary tubing, and saturated potassium chloride solution from a calomel half-cell seeped through the filter tip ( $0.16 \text{ ml flow/hr}$ ). Both these substances entered the water stream and were carried away in the effluent from the block. Voltages were applied across these electrodes and measured with a pH electrometer equipped with a voltage divider to increase its range. Electrical currents were measured with a moving coil, reflecting galvanometer. Water flow was controlled to within  $1/10 \text{ ml/min}$  by use of the Mariotte's flask principle in a constant-temperature room. The flow was  $5 \text{ ml/min}$  in the experiments presented in Fig. 1.

The usual procedure requires that the apparatus be

calibrated at a single voltage. The 1-v Weston cell was used by Petering and Daniels (2), Manning (1), and Spoor (3). Although this is a convenient source of constant potential difference, it places the experiment at a region of the polarogram where the slope is steep (Fig. 1). Slight voltage changes in the system could thus cause considerable variations in the current at a constant level of oxygen. This hazard could be avoided by working at 1.6 v, where the current is relatively independent of the voltage over a wide range. Beckman (4) used the first oxygen reduction wave at 0.42 v, but the sensitivity to oxygen in this range is about half that at 1.6 v, and it is characterized by a very pronounced maximum (5).

The limiting currents (at 1.6 v applied) for each of the oxygen concentrations of Fig. 1 are shown in Table 1. There was a linear relationship between oxy-

TABLE 1

LIMITING CURRENTS OF VARIOUS CONCENTRATIONS OF OXYGEN IN LAKE WATER

| Oxygen (ppm) | Limiting current |                   |
|--------------|------------------|-------------------|
|              | $\mu\text{a}$    | $\mu\text{a/ppm}$ |
| 1.43         | 0.46             | 0.32              |
| 5.78         | 2.63             | 0.46              |
| 8.62         | 3.89             | 0.45              |
| 9.63         | 4.20             | 0.44              |

gen concentration and limiting current at 5.78-9.63 ppm with a slope of 0.44-0.46  $\mu\text{a/ppm}$ . The ratio 0.32 at 1.43 ppm suggests a departure from linearity between low and high levels of oxygen. Variations in the residual current, however, could account for this discrepancy (5). A polarogram by Kolthoff and Lingane (5) indicated a sensitivity for oxygen of 0.67  $\mu\text{a/ppm}$ , or about 45% greater than that obtained in the present experiments.

The great sensitivity of the method is evident in the above data. However, troublesome variations made the present application unsuitable for critical determinations of respiration. A typical deflection is shown in the dotted line of Fig. 1. At constant oxygen level (8.6 ppm) the galvanometer readings over the oxygen range were depressed as much as 3 cm from the usual values coincident with an observed increase in the mercury drop rate. This variation was associated with the diffusion of oxygen at the mercury surface, for it had its effect only below 2.2 v.

A supporting electrolyte was not used in the experiments; hence the wave height may have varied with the electrolyte content of the water at constant oxygen concentration. The seepage of potassium chloride from the calomel half-cell may be one source of trouble, although Spoor (3) recommended such a system to abolish variations in the limiting current at constant voltage.

Petering and Daniels (2) hoped to cancel out fluctuations in the readings that were due to temperature and accidental variations in the amounts of iron and

other easily reducible substances by determining the current at 1.0 and 0.1 v. The oxygen concentration was then related to the difference between the currents flowing at these two voltages. However, in the present experiment (Fig. 1) there was a change of 12 cm in the galvanometer deflection at 0.1 v when the oxygen concentration was varied from 1.4 to 9.6 ppm. Also, the half-wave potential of the first oxygen wave may be expected at about -0.05 v vs. the saturated calomel electrode (5). The 0.1 v reading is thus well above the decomposition potential for oxygen and should not be used to represent the residual current—that current which would flow in an oxygen-free solution at the working voltage.

The temperature coefficient of the process at 1.0 v was 1.3%/C degree at 4.3 ppm, and 1.6% at 8.0 ppm of oxygen. The latter value is equal to that applied by Manning (1), and both are within the range 1.3-1.6/degree quoted by Kolthoff and Lingane (5). Temperature and other variations that are a function of the total current obviously cannot be accounted for by the mere difference between the limiting and residual currents. A proportionality factor would have to be introduced. Frequent standardization against an air-saturated test solution (5) may yield more favorable results.

The dropping mercury electrode can be used to measure oxygen in flowing, unmodified lake water. However, the many variables which must be controlled or accounted for to obtain precise results render the method exceedingly difficult to apply in this manner.

#### References

1. MANNING, W. M. *Ecology*, **21**, 509 (1940).
2. PETERING, H. G., and DANIELS, F. J. *Am. Chem. Soc.*, **60**, 2796 (1938).
3. SPOOR, W. A. *Science*, **106**, 421 (1948).
4. BECKMAN, P. *Chemistry & Industry*, **26**, 791 (1948).
5. KOLTHOFF, I. M., and LINGANE, J. J. *Polarography*. New York: Interscience (1941).

Manuscript received September 30, 1952.

## Relationship of Colloids to the Surface Tension of Urine

Robert A. Ravich

*Institute of Applied Biology, Brooklyn, New York*

The idea that colloids in the urine are of major importance in preventing precipitation, agglomeration, and conglomeration of crystalloids from a supersaturated solution has recently aroused renewed interest through the work of Butt and his associates. Ebstein in 1884 first suggested that the urinary crystalloids remain in the dissolved state through the protective action of the urine colloids (1).

Butt appears to have been the first to suggest that this protective action might be applied to the treatment of renal lithiasis (2). He found that the number of colloid particles visible in the urine when examined

under the ultramicroscope is increased by the administration of hyaluronidase (3), and presented clinical evidence which indicated that hyaluronidase may effectively delay or stop the formation of new stones in patients who recurrently form stones (4).

Butt, Hauser, and Seifter (4) attribute the protective effect of the colloids to the influence they exert upon the surface tension of the urine. They found that hyaluronidase both increased the number of colloids and decreased the surface tension of the urines of patients treated with this enzyme.

During the past six years the surface tension values of serial urine specimens of a large number of patients have been routinely followed in this laboratory. A capillary tube calibrated to permit direct reading of the surface tension in dynes/cm has been employed. The design, calibration, and use of this urotensimeter have been described by Revici (5). Determination of the surface tension with this instrument is as simple as the reading of a clinical thermometer, and can be made a routine part of any urinalysis.

The urinary excretion of tensioactive agents has been found to be altered in various disease states (6), but the exact nature of these tensioactive substances is unknown. It therefore seemed worth while to determine whether the urinary colloids could be directly related to the surface tension, as suggested by Butt, Hauser, and Seifter (4), using the urotensimeter for this purpose.

Consecutive urine specimens from 50 persons were used. A drop of urine was placed on a clean glass slide, covered by a clean cover glass, and examined under the ultramicroscope. The colloid particles are readily identified as tiny pinpoints of light possessing erratic Brownian motion. The number of these particles in an average high dry field ( $\times 440$ ) was estimated, and the specimens were divided into groups according to the number of colloid particles present.

The surface tension of the same urine specimens was determined, using the urotensimeter. The tip of the calibrated capillary tube was introduced into the specimen, and the urine was drawn up above the top mark of the scale by gentle mouth suction and then expelled twice in order to clear the lumen of the previous specimen. Urine was then drawn up a third time to the same level, and the tube removed from the specimen and read. The level at which the descent of the column first came to a stop or where the rate of descent was distinctly slowed was recorded as the surface tension of the specimen in dynes/cm (5). The urine specimens were divided into groups according to the surface tension values found.

When the urine specimens were grouped according to the number of colloids rendered visible under the ultramicroscope, 4 specimens showed no colloid activity, 11 showed 1-5 colloid particles/high power field, 5 had 6-20 particles, 15 had 21-40 colloid particles, and 15 had more than 40/high power field.

When the urines were divided on the basis of the surface tension values of the specimens, 21 had sur-

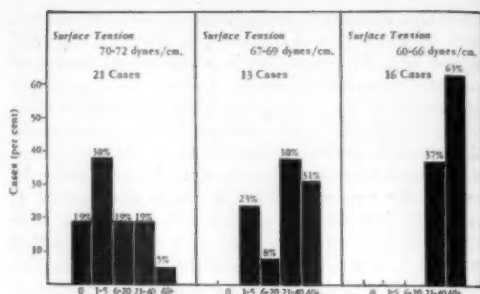


FIG. 1. Number of colloids per high power field.

face tension values of 70-72 dynes/cm, 13 had values of 67-69 dynes/cm, and 16 had values below 66, ranging as low as 60 dynes/cm.

In Fig. 1, the correlation between the number of colloid particles/high power field and the surface tension of the urine is shown graphically. It can be seen that the urines having surface tension values of 66 dynes/cm or less contained 21 or more colloid particles in each high power field. Conversely, those urines which showed no colloid activity had surface tension values of 70-72 dynes/cm. The urine specimens with 1-20 colloid particles/high power field all had surface tension values above 67 dynes/cm. However, some urine specimens with a high level of colloid activity (i.e., more than 20/high power field) had surface tension values of 70-72 dynes/cm.

Our findings indicate that there is a correlation between the number of colloids present and the surface tension of individual urine specimens. Generally, urine specimens exhibiting a high degree of colloid activity have low surface tension values, whereas specimens in which little or no colloid activity is apparent have high surface tension values. Similar findings apparently led Butt, Hauser, and Seifter (4) to attribute the protective action of the urinary colloids to an influence exerted by the colloids upon the surface tension of the urine.

The finding that a significant number of urine specimens in the group studied had high surface tension values, even in the presence of a large number of colloid particles, indicates that the colloids do not actually determine the surface tension of the urine. Thus, although a general inverse relationship exists between the number of colloid particles and the surface tension of the urine, it appears that the surface tension is not determined by the number of colloids present, or vice versa.

Butt and his associates observed that administration of hyaluronidase produced a higher degree of colloid activity and a reduction in the surface tension of the urine of treated patients (4). Our findings indicate that the apparent retardation of stone formation observed in the patients treated with this enzyme might have been due to the reduction in surface tension, the increase in colloid activity, or perhaps to some other unknown factor fundamental to both.



In pursuing this aspect of the stone problem further, it would be of interest to study the surface tension values along with the degree of colloid activity and to determine whether some common factor influences both of these. In the past, surface tension determinations have been difficult to perform, requiring considerable time, intricate instruments, and the solution of complicated formulas. The simple urotensiometer designed by Revici (5) obviates these difficulties and makes it possible to perform accurate determinations on all urine specimens as a routine procedure. Preliminary studies have indicated that urine surface tension determinations may increase our knowledge of the mechanisms involved in stone formation and may be helpful in the management of renal lithiasis (7).

#### References

1. ERSTEIN, W. *Die Natur und Behandlung der Harnsteine*. Wiesbaden: J. F. Bergmann (1884).
2. BUTT, A. J. *J. Florida Med. Assoc.*, **37**, 711 (1951).
3. —, *J. Urol.*, **67**, 450 (1952).
4. BUTT, A. J., HAUSER, E. A., and SEIFTER, J. *Calif. Med.*, **78**, 123 (Mar. 1952).
5. REVICI, E. In press.
6. —, To be published.
7. RAVICH, R. A., and RAVICH, A. To be published.

Manuscript received September 26, 1952.

### Production of Folinic Acid from Folic Acid by *Lactobacillus casei*<sup>1</sup>

T. J. Bond

Department of Chemistry, Baylor University, Waco, Texas

It has been reported (1) that naturally occurring folinic acid (citrovorum factor) appeared, on the basis of microbiological assay, to be some fifteen times as effective as folic acid in competitively reducing the toxicity of x-methyl folie (2) acid for *Lactobacillus casei* in a defined synthetic medium. Accumulated evidence (i.e., 3-6) appears to place folinic acid as at least one metabolically active form of folic acid and makes it highly probable that dietary folic acid is converted to folinic acid, and that the latter active principle is the naturally occurring factor. Further support for this belief is found in noting that the methods employed in the isolation of folic acid from natural sources are sufficient to convert folinic acid to folic acid (7).

In this laboratory enhanced folinic activity has been observed in the livers of normal, healthy rats growing on an adequate diet supplemented with added folic acid. In a comparative fashion, it was thought that investigation of possible biosynthesis in microorganisms would be profitable.

During experiments designed to ascertain the possible metabolic function(s) of folinic acid, the biosynthesis of this factor by each of several microorganisms was observed. *L. casei* was selected as the organism for

<sup>1</sup> Studies on possible nutritional significance of folinic acid, of which this paper is a part, are supported in part by the Williams-Waterman Fund of the Research Corporation.

TABLE 1  
FOLINIC ACID CONTENT OF *Lactobacillus casei* MEDIA

| Folic acid<br>( $\mu\text{g}/50\text{ ml}$ ) | Folinic acid*<br>( $\mu\text{g}/50\text{ ml}$ ) |                      |
|--|---|----------------------|
|  | Uninoculated<br>medium                          | Inoculated<br>medium |
| 10   | 00  | .1                   |
| 30   | 00  | 1.8                  |
| 100  | 00  | 23.0                 |
| 300  | 00  | 28.0                 |
| 1000   | 00  | 50.0                 |
| 3000   | 00  | 90.0                 |

\* Amounts based upon folinic acid SF (synthetic folinic acid) as the standard.

the purpose of reporting the production of folinic acid by a microorganism growing in a defined synthetic medium.

An enriched medium, essentially that of Rogers and Shive (8), but modified to contain purines and varying amounts of folic acid, was prepared in double strength and diluted before sterilization with an equal volume of phosphate buffer at pH 7. A small portion of each test (10 ml) was removed from uninoculated blanks as medium controls. The remainder (40 ml) in each case was heavily seeded with 1 ml of a highly turbid 10-ml saline suspension of saline washed cells of an actively growing 24-hr culture of *L. casei*, ATCC No. 7469, carried routinely on glucose-yeast-agar in this laboratory.

After 24 hr incubation (static culture) at 37° C, the cells were removed by centrifugation, the medium was neutralized with sodium carbonate, and the relative folinic acid content in each medium, inoculated and uninoculated, was estimated microbiologically, using *Leuconostoc citrovorum*, ATCC 8081, as the test organism and synthetic folinic acid-SF<sup>2</sup> as the standard (Table 1). The assay medium was essentially that described by Snell *et al.* (9) but was modified to contain asparagine, folic acid, pyridoxine, and inositol.

All assays were incubated 16-18 hr at 37° C. Graded growth responses were measured turbidimetrically with a Klett-Summerson photoelectric colorimeter, using light filter No. 54.

At the conclusion of the incubation period it was observed that the pH of the medium containing *L. casei* was moderately acid in spite of the presence of the added phosphate buffer. Since folinic acid activity is destroyed by mild acid hydrolysis (10), the amount of this factor remaining in the medium (Table 1) at the conclusion of the incubation period will not necessarily present a true picture of the actual conversion of folic acid to folinic acid by this microorganism. However, these data will serve to illustrate that such a transformation is accomplished.

Samples of medium containing folinic acid activity were compared bioautographically with synthetic folinic acid, employing paper chromatograms developed

<sup>2</sup> Supplied through the courtesy of E. I. Lilly and Company, Research Laboratories, Indianapolis, Indiana.



with 2,6-lutidene. Two areas of growth were observed. One area compared favorably with synthetic material while the other area indicated a faster moving active principle.

No effort was made in this experiment to obtain maximum release of folinic acid either from the cells or from whatever bound form in which the factor may exist.

#### References

1. BOND, T. J., et al. *J. Am. Chem. Soc.*, **71**, 3852 (1949).
2. WILLIAMS, R. J., et al. *The Biochemistry of the B Vitamins*. New York: Reinhold, 575 (1950).
3. KLINE, I. T., and DORFMAN, R. I. *Proc. Soc. Exptl. Biol. Med.*, **76**, 203 (1951).
4. NICHOL, C. A., and WELCH, A. D. *Ibid.*, **74**, 52 (1950).
5. FLYNN, E. H., et al. *J. Am. Chem. Soc.*, **73**, 1979 (1951).
6. SHIVE, W., et al. *Ibid.*, **72**, 2817 (1950).
7. MAY, M., et al. *Ibid.*, **73**, 3067 (1951).
8. ROGERS, L. L., and SHIVE, W. J. *Biol. Chem.*, **172**, 751 (1948).
9. SNELL, E. E., GUINARD, B. M., and WILLIAMS, R. J. *Ibid.*, **143**, 519 (1942).
10. BARDOH, T. J., et al. *J. Am. Chem. Soc.*, **71**, 3852 (1949).

Manuscript received October 2, 1952.

### The Life History of *Echinoparyphium flexum* (Linton 1892) (Dietz 1910) (Trematoda: Echinostomidae)

Haig H. Najarian

Department of Zoology, University of Michigan,  
Ann Arbor<sup>1</sup>

Linton (1) described the trematode *Distomum flexum* found in the intestine of the black scoter, *Oedemia americana*, from Yellowstone National Park. Dietz (2) incorporated the species in the genus *Echinoparyphium*. McCoy (3) obtained the adult experimentally by feeding chicks metacercariae from the snail, *Planorbis*? (= *Helisoma*) *trivolvis*, collected from Romana Lake, Missouri. Although his attempts to hatch the trematode egg were unsuccessful, he was able to obtain cercariae in 9 weeks by placing eggs in a small aquarium with laboratory-reared *Physa integra*. Najarian (4) found the metacercaria in the kidneys of several species of frogs in the vicinity of Ann Arbor, Michigan.

The life cycle of the worm has been experimentally established in this laboratory, and all the stages have been studied. The chick was used as the experimental definitive host. The natural definitive host was found to be the blue-winged teal, *Anas discors*. The small intestine of two of the eight ducks shot in a wood-spool area six miles west of Ann Arbor, Michigan, contained the adults of *E. flexum*.

The natural snail host in the area studied was *Lymnaea palustris*. Of the 3755 specimens collected and individually isolated, 83, or 2.2%, showed infection with the cercaria. The percentage of infected snails was small and ranged from 1.6 to 2.4 from April through October 1952. A single infected snail

sheds 900-1300 cercariae within a 24-hr period. The bulk of shedding takes place between 1:00 P.M. and 4:00 P.M.

The metacercaria was found in nature both in the kidneys of frogs and tadpoles and in the kidney and heart of *Lymnaea palustris*. The cysts were found in the following species of frogs: 108 *Rana sylvatica*, 75% infected; 9 *Hyla crucifer*, 88% infected; 8 *Pseudacris migrata triseriata*, 25% infected; 7 *Rana pipiens*, 42% infected; 41 *Rana clamitans*, 14% infected.

Young adults of *R. pipiens* and *R. sylvatica*, reared in the laboratory from the egg stage, could not be infected by exposure to the cercariae. Tadpoles of the same species were easily infected. The metacercaria found in the kidneys of frogs in nature is apparently the result of the cercaria entering the tadpole and remaining in the kidney until metamorphosis is completed.

Experimentally, the cercaria encysts in the snails *Lymnaea palustris*, *Gyraulus parvus*, and *Physa gyrina*. In all cases the cysts are infective within 24 hr.

The feeding of the infective metacercariae to chicks was shown to give only a 1.1-1.6 yield of adult worms of *E. flexum*.

The eggs leave the uterus of the worm in an unclevated condition. In the laboratory they were incubated in aerated tap water at room temperature from May through August 1952 and were shown to hatch in 10-14 days. The ciliated epidermal plates of the miracidium were studied by the silver nitrate technique and were found to have the formula 6-6-4-2 = 18 plates. The two plates of the fourth tier are lateral and not dorso-ventral as in the genus *Echinostoma*. This feature is apparently characteristic for the genus *Echinoparyphium*.

The miracidium penetrates young specimens of *L. palustris* and within 7 hr is found within the heart of the snail. There, within 24 hr, the miracidium transforms into a sporocyst stage. Johnson (5) believed that the miracidium of *E. revolutum* metamorphoses directly into a redia. This study supports the results of Mathias (6), Rasin (7), and Churchill (8), all of whom observed sporocysts in their echinostome studies.

Mother rediae developed from the sporocysts and were first seen in the snail heart at 9 days. At 10 days they were found in the lumen of the heart and were extremely variable in shape. They were shown to be the migratory stage of the mother rediae. In no cases were daughter rediae observed in the heart of the snail.

The mother rediae migrate, apparently, via the snail's circulatory system. They were found at 10-12 days in the digestive gland and ovotestis, where they produce large numbers of daughter rediae.

The daughter rediae are avid eaters, and in both natural and experimental infections the gut was found to be filled with orange-colored material of the snail's

<sup>1</sup> Contribution from the Department of Zoology, University of Michigan, under the direction of Dr. A. E. Woodhead.

digestive gland. In many snails of natural infection the digestive gland was found to be reduced to about one-fifth of its usual mass. In one specimen, which died shortly after being brought into the laboratory, 1647 daughter rediae, by actual count, were removed from the snail.

Recognizable cercarial embryos were first seen within the daughter rediae at 33 days. They are fully formed when they leave via the birth pore of the redia. After entering the lung cavity, the cercariae emerge from the snail via the respiratory aperture. The total time from exposure of the snails to the miracidium to the first emergence of the cercariae from the respiratory aperture was 40-46 days.

Details of the life cycle with systematic considerations will be published later.

#### References

1. LINTON, E. *Proc. U. S. Natl. Museum*, **15**, 87 (1892).
2. DIETZ, E. *Zool. Jahrb., Suppl.*, **12**, 265 (1910).
3. MCCOY, O. R. *J. Parasitol.*, **14**, 207 (1928).
4. NAJARIAN, H. H. *Ibid.*, **38**, (4), Suppl. 38 (1952).
5. JOHNSON, J. C. *Univ. Calif. (Berkeley) Publ. Zool.*, **19**, 335 (1920).
6. MATHIAS, P. *Ann. sci. nat., Bot., et Zool.*, **10**, 289 (1927).
7. RASIN, K. *Spisy Vysoké. Školy. Zool. Brno. C.S.R.*, **19**, 1 (1933).
8. CHURCHILL, H. M. *J. Parasitol.*, **38**, (6), Suppl. 27 (1950).

Manuscript received October 16, 1952.

## The Cultivation of Hydra Under Controlled Conditions

W. F. Loomis

The Loomis Laboratory, Greenwich, Connecticut

Despite the classical studies that have been carried out on the regenerative capacities of hydra, as well as their extensive use in teaching courses, no reliable method has been available for their continued culture under controlled conditions, a fact that may explain their having been neglected to date as experimental animals.

Previous methods of culturing hydra have required the use of such variable and uncontrolled media as pond (1-5) and aquarium (6) water and, in addition, have needed subcultures of the water-flea *Daphnia* or other crustacean to provide the living food needed by all species of hydra. Unfortunately, present methods of culturing *Daphnia* are as unsatisfactory as those for culturing hydra; one recent publication has stated (7): "So far as we have been able to determine by experimentation, there has been no 'sure-fire' method discovered of keeping a culture of *Daphnia* permanently in the laboratory." The usual technique is to maintain four or five cultures in large wooden tubs, or barrels, so that there is a good chance that at least one culture will contain numerous *Daphnia* at any given time.

Even with an adequate supply of living food available, culturing hydra has been difficult. Hyman has stated (4): "The great difficulty in the continuous

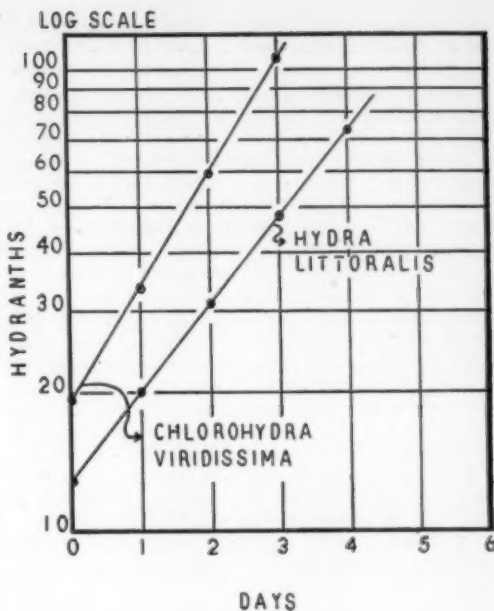


FIG. 1. Logarithmic increase of hydranths in two species of hydra grown under controlled conditions at  $20 \pm 0.5^\circ \text{C}$  (provisional identification of hydra *Littoralis*).

culture of hydra is the occurrence of the phenomena of 'depression.' In spite of every care, hydra will pass into this state at intervals and, unless prompt measures are taken, will die out. In depression, column and tentacles fail to expand, the animal ceases to feed, shortens to a stumpy appearance, and finally disintegrates from the tips of the tentacles aborally."

The method of culturing hydra described here avoids depression by controlling both the medium and the food supply of the hydra. In place of pond water, a chemically defined solution is utilized. In place of *Daphnia* cultures, the dried and stable eggs of the brine shrimp *Artemia* are used as a source of living crustacea. As these dried eggs are viable for years, they may be hatched on schedule in reproducible batches of any desired size.

By utilizing the technique described below, rapid logarithmic reproduction (asexual) has been observed (Fig. 1), and thousands of hydra obtained daily with a minimum of effort. All the species studied to date have grown well under these conditions,<sup>1</sup> and depression has been entirely avoided.

1) Brine shrimp eggs<sup>2</sup> are hatched serially at room temperature on a 48-hr schedule, the daily routine

<sup>1</sup> Purchased hydra are often received in a state of depression. On receipt they should be placed singly in test tubes and fed and changed daily until actively budding. After a clone of 10-20 has been formed, they may be transferred to larger vessels.

<sup>2</sup> Brine shrimp eggs are available in quantity from aquarium stock companies.

consisting of dusting a quarter of a teaspoonful (0.7 g) of *Artemia* eggs on the surface of 500 ml of 3.5 g/l NaCl solution contained in a large flat Pyrex dish (Corning 3-qt utility dish). It is convenient to prepare this salt solution by diluting a stock solution of saturated NaCl solution a hundredfold with tap water.

2) Forty-eight hours later, the hatched larvae are collected in a fine-mesh net, washed with L solution (see below), and added to the cultures of hydra. Living larvae can be separated from unhatched eggs by phototropic migration.

3) The cultures of hydra are grown at room temperature in similar shallow dishes in "Littoralis (or L) solution" of the following composition: 0.35 g/l NaCl; 0.07 g/l  $\text{CaCl}_2$ ; 0.01 g/l  $\text{NaHCO}_3$ . This solution should be made up in either distilled or deionized water, as tap water is toxic to hydra in most localities. In practice, it is convenient to make up this solution by filling a gallon jug with water from a laboratory demineralizer after having added 10 ml each of the following two stock solutions: (1) 133.0 g NaCl, 26.6 g  $\text{CaCl}_2$ , demineralized water to 1 l; (2) 3.8 g  $\text{NaHCO}_3$ , demineralized water to 1 l.

4) The L solution in which the hydra are grown should be changed within 24 hr of feeding them with

brine shrimp larvae. For this, it is only necessary to decant the old and fouled solution, replacing it with new, as most of the hydra will be found to adhere tenaciously to the glass bottoms of the dishes. The few hydra that are decanted may be easily collected by swirling the old solution in round containers; they will be found to collect in the center.

5) When active increase is not desired, hydra may be left in clean L solution without feeding for several weeks at room temperature, or for several months in a refrigerator. Such stored specimens will begin to bud once again approximately 48 hr after being returned to room temperature and fed daily with an excess of brine shrimp larvae.

# References

1. HYMAN, L. H. *Trans. Am. Microscop. Soc.*, **49**, 322 (1929).
2. EWER, R. F. *Proc. Zool. Soc.*, **117**, 207 (1947).
3. HADLEY, C. E., and FORREST, H. *Am. Museum Novitates*, No. 1423 (1949).
4. NEEDHAM, J. G. *Culture Methods for Invertebrate Animals*. Ithaca, N. Y.: Comstock (1937).
5. BRIEN, P. *Eull. soc. zool. France*, T. LXXVI, 277 (1951).
6. TURNER, C. L. *Biol. Bull.*, **59**, 285 (1950).
7. *Turtos Service Leaflet No. 39*. Chicago: General Biological Supply House.

Manuscript received October 3, 1952.

## An Ultrafilterable Growth Factor for Tissue Culture<sup>1</sup>

Sheldon Rosenberg and Paul L. Kirk

Department of Biochemistry, University of California, Berkeley

The use of tissue cultures as a means of screening chemotherapeutic agents and in studying fundamental problems of growth has increased considerably in the past few years. Attempts have been made to standardize the technique and define the results in specific chemical terms (1, 2). A purely synthetic medium, comparable to those used in bacterial cultures, would provide an ideal solution. Attempts at synthesizing a medium empirically have been made (3, 4) but the results have not been completely satisfactory.

Fischer and his co-workers (5) made a start toward finding factors, both in chick embryo extract and in other natural sources, which could simplify the present day media. Their work resulted in the use of some partially purified, but chemically undefined, fractions which could be substituted for embryo extract. In this laboratory, it is believed that an analytical approach to the problem is more promising than a synthetic approach. We have been able to obtain a fraction from the ultrafilterable portion of chick embryo extract

which has been partially analyzed by paper chromatography and ionophoresis. It showed the presence of about five components.

Embryo extract prepared as previously described (6) was ultrafiltered. The ultrafiltrate was lyophilized and the powder was extracted with 70% ethyl alcohol. The alcohol extract was then passed through a column of Amberlite IR-120 in the hydrogen form and was washed through with two volumes of alcohol. The effluent was neutralized to pH 7.0 and evaporated to a volume of 1-3 ml. The solution was made to the original volume with distilled water. This fraction contained about 3% of the original ultrafilterable nitrogen and was as active as untreated ultrafiltrate when added back to thoroughly dialyzed embryo extract proteins. The method of culturing and defining the results were those developed in this laboratory by Signorotti, Hull, and Kirk (2, 6). At present the three ninhydrin positive materials and two ultraviolet absorbing materials which comprise this fraction are being identified.

# References

1. DAVIDSON, J. N., and LESLIE, I. *Nature*, **165**, 49 (1950).
2. HULL, W., and KIRK, P. L. *J. Gen. Physiol.*, **34**, 81 (1950).
3. WHITE, P. R. *Growth*, **10**, 231 (1946).
4. MORTON, H. J., MORGAN, J. F., and PARKER, R. C. *J. Cellular Comp. Physiol.*, **33**, 389 (1951).
5. ASTRUP, T., FISCHER, C., and VOLKERT, M. *Acta Physiol. Scand.*, **9**, 134 (1945).
6. SIGNOROTTI, B., HULL, W., and KIRK, P. L. *J. Gen. Physiol.*, **33**, 315 (1950).

Manuscript received October 8, 1952.

<sup>1</sup> Supported in part by a grant from the National Cancer Institute of the National Institutes of Health, USPHS.

# Comments and Communications

## Science Teaching in the Secondary Schools

A VERY important problem in secondary science teaching in addition to those mentioned by Professors Schriever (*SCIENCE*, 115, 96 [1952]) and Watson (*SCIENCE*, 116, 261 [1952]) is how to interest high school students in taking the available mathematics and physical science courses. Today we find that, even in large high schools with competent staffs and adequate laboratory facilities, the enrollment in these courses is relatively small. Professor Schriever points out the same problem when he states that the U. S. Naval Academy had to forego the requirement of high school physics for its prospective candidates. How often, however, have college science instructors heard their students remark how they wished that they had taken more high school mathematics and science. It is certain that many promising young men and women have been lost to the scientific professions because of this lack of background.

Here, of course, we can hear the objection that the job of the high schools is only in part college preparatory, and thus the importance of mathematics and science is often minimized by some public school administrators. The answer to this objection was well given by Professor Schriever, that the secondary schools take a lead from the colleges by requiring of all students good general education courses in the physical sciences. These courses should include laboratory work and elementary mathematical treatments of the subject matter.

My big question, however, is: Why do we not guide our high school students better in their course selection? The high school student naturally takes the easiest way out and most often chooses not to take the courses under question, because they already have a reputation of being hard and requiring more work. If the parents also fail to exercise their influence through lack of understanding of the problem, the student loses out on one of the most important phases of his education, which would provide him with a better appreciation of his natural environment and of every day living in a technological age.

If we believe in "life adjustment education," as one of the latest pedagogic phrases goes, why do we not require our students to take these courses? How do we expect immature high school students to make their own correct choices when nearly every department in institutions of higher learning requires the more mature college students to follow rather closely prescribed curricula, which allow in most instances for only a few elective courses through their whole college career?

I believe we educators too often underestimate the capabilities of our students, and on requiring more mathematics and science in the high schools we would find not only that students could manage these courses but that they would even greatly enjoy them. The re-

sults would be well worth the extra effort, for it would lead to a laity better informed on scientific questions and at the same time help to fill the sadly depleted ranks of scientific personnel.

ANTON POSTL

*Oregon College of Education, Monmouth, Oregon*

## Employment and Education Fair in Science

At the initiation of the New York Branch of the American Association of Scientific Workers a job and educational conference was held at the New Lincoln School on Saturday afternoon, September 13. Sponsored jointly by the AAScW, the American Council on Human Rights and Committee to End Discrimination in Science and Health, the fair has made a definite contribution toward the achievement of equal opportunities for Negroes in science.

Alexander Sandow, professor of biophysics at New York University, addressed the opening session. "The very fact that such outstanding men as Benjamin Bannaker, James Derham, Elijah McCoy, Jan Matzeliger, Granville T. Woods, Ernest E. Just, Charles Drew, and George Washington Carver have arisen," said Dr. Sandow, "serves to emphasize the tremendous loss which science in our country has suffered through the practice of discrimination." He pointed out that as long as discrimination exists there can be no true democracy and called upon scientists to work to bring about an end to this practice in their own fields. Miss Valjeanne Taylor of the American Council on Human Rights and Cuthbert Pitter of the Committee to End Discrimination in Science and Health were co-chairmen of the meeting.

Some 20 outstanding Negro and white scientists participated as consultants on the panels held in the fields of physics, biological sciences, chemistry, psychology, and engineering. Among the panel consultants were I. Fankuchen, professor of crystallography at the Polytechnic Institute of Brooklyn, Peter Bergmann, professor of physics at Syracuse and at the Polytechnic Institute of Brooklyn, Harry Grundfest, associate professor of Neurology at Columbia University, and Lester Florant, research engineer at the Allen B. Dumont Laboratories.

The most important part of the fair was that in which the panels were in session. Here Negro scientists and prospective scientists sought council in the panels on questions dealing with job and educational opportunities in science. Despite serious individual problems the registrants seemed most concerned with developing general approaches to the problem of discrimination. A Negro engineer emphasized the need for a more positive approach which laid stress upon the present professional and scientific activities of his people as a means of encouraging young people to enter the field as a career. Other speakers urged white and Negro scientists to take the offensive in opening



up new job opportunities where they do not now exist.

Plans have been made to continue the work begun by the fair. A continuations committee which includes many scientists among its members has been set up for this purpose.

JOHN F. CODINGTON

*Department of Biochemistry, Columbia University*

### Science versus Administration in Certain U. S. Foreign Aid Efforts

IT WAS to be expected that the foreign aid programs would receive the immediate and critical attention of the new administration. The American people perhaps do not realize what a powerful position the various foreign aid agencies, as a group, have occupied in the nation's international relations. The influence which our economic aid activities have had on those relations in the separate countries that have received the aid should not be underestimated.

There certainly should be little opposition to the idea, *per se*, that certain kinds of aid ought to be extended by the United States to various other nations. Our foreign aid efforts to date have contributed much toward the postwar development of some countries. It must be realized, nevertheless, that a particular program in whatever country is no better than the management of it or the soundness of its purpose.

The scientists of our own country are indispensable to the government's foreign economic aid efforts. Their interest in the programs has been essential to the development of the American foreign aid activities. The confidence of scientists in the government's aid efforts, however, can be based only upon the soundness and the effectiveness of the various programs. The program accomplishments of an American foreign economic aid organization in any country can be limited by the quality of the local administration of the program and by the support of that administration in Washington, regardless of the collective efforts of the technical field staff.

The contributions of science in the foreign aid efforts, therefore, can be greatly restricted, and the personnel affected by such restrictions can experience professional humiliation, whenever the administration of any particular technical aid program is placed by the government in the hands of persons who are professionally unqualified to administer such a program. The Mutual Security Agency (MSA) general agriculture program in Thailand has been representative of such a situation.

One of the ultimate purposes of any agricultural development program is to make those developments directly beneficial to the farmers. Americans, however, are foreigners to the other peoples of the world; and our intentions and activities in any country outside our own will usually be held in doubt by the rural populations of that country until we have properly demonstrated, in the course of time and by good works, just what our intentions are. Therefore, an all-

out "immediate impact" agricultural aid campaign at the "grass-roots" or "village level" in an economically undeveloped and slow-moving country is a risky matter in foreign relations.

It must also be remembered that, among other important considerations, no occupation brings a man closer to his religion than farming. Many Asiatic rural customs and religious beliefs are still closely associated with farming practices. Unrestrained tampering with this way of rural life through the importation and distribution of *untested* fertilizers, mechanical equipment, seeds, and ideas on farming practices is inviting possible outcomes we have not planned. Agricultural aid efforts, furthermore, at the "grass-roots" in a modern national economic development program soon lose impetus, when not supported by agricultural research and training programs and by certain agricultural economic considerations.

Certain countries in the Asiatic regions (such as Formosa, Indonesia, Japan, and to a limited extent, China and the Philippines) have carried on agricultural research as far back as the early part of this century. Our technical people probably have profited from this work in rendering aid to those countries. Such a stockpile of technical knowledge has not been available to our technical personnel in other areas.

Thailand has not been economically desperate, but the development of the country depends considerably on foreign technical guidance and on a proper amount of imported equipment. Thailand is over 90 per cent agricultural, yet the accumulation of scientific data and the application of the methodology of science to agricultural problems are only in the beginning stage. Original plans for American economic aid to the Kingdom, therefore, stressed the development of a sound general agricultural program on a long-term basis. This scheme provided for agricultural research and training programs which would, in turn, support a later extension service to the rural population. Only rarely could Thai farmers be properly advised on the basis of U. S. agricultural conditions or data. Only in a very limited manner could American efforts to aid general agriculture even attempt an "immediate impact" as of 1950-51.

The higher level MSA-Thailand administrative support of a soundly based general agricultural aid program for the Kingdom began to be withdrawn in late 1951. A confusion of new government "policies" overwhelmed the field operations. Reflections were cast by the program administrators upon the professional competence of the agricultural scientists, as a subtle campaign seemed to be in effect against the current agricultural technical activities and against the leaders of the research and training program.

The unethical administrative tactics employed against members of the agricultural technical staff make it interesting to speculate about just what was going on—and for that matter still is. The refusal of the leaders of the research and training program to cooperate in the proposed "give-away" and the "dramatic" tactics in the field operations only widened the



break in relations between the MSA and the scientists in this mission. The effectiveness of the efforts of certain staff members in the general agriculture program diminished rapidly.

In late January, 1952, the director of the agricultural training program finally requested his release from the Thailand mission. Soon afterwards, the livestock development specialist was asked to resign. The first scientist returned to the MSA in Washington in February, 1952, where his appeal was laid aside, after a farcical "hearing." Other divisions of the MSA in Washington were instructed not to secure the services of this scientist, and the director of his own division in the MSA would not confer with him after his return. The second staff member was not returned to Washington, where he might have attempted an appeal of the action taken against him.

Simultaneous with the departure from Thailand of the two men just mentioned, the Mutual Security Agency in Washington requested, through the Office of Foreign Agricultural Relations, the recall from service in Thailand of a third member of the general agriculture technical staff. This scientist's monumental work of fifteen years on the soils of Thailand has been a basis upon which much of the agricultural development of the Kingdom is now being laid. His thirty-odd years of outstanding research in the tropical areas of the world were recognized in the 1950 award of the American Geographical Society's Livingston medal. His recall from Thailand was halted by events in Bangkok which, to say the least, were anything but a master stroke in good relations between the Siamese and American peoples.

The Mutual Security Agency's only explanation for its actions regarding these men was that they could not get along with the Thai people. In Bangkok, abusive criticisms were heaped on them by the Chief of the MSA mission relative to their services in Thailand. It is interesting to note, however, that less than four months prior to these events, the Thai government had requested of the MSA that the soil scientist in question be appointed to fill the newly vacated directorship of the agricultural division in the mission, and it has recently asked him to assume responsibilities in Thailand's agricultural ministry. Furthermore, a second member of this trio was largely responsible for pioneering the new government vocational agriculture movement in another Asiatic country. Recent translations of his writings for the general population on home food production have already gone well into the third printing.

The technical work of the general agriculture program of the MSA mission in Thailand has continued to experience pressure against it. Not one of the original leaders of the project has completed his full term of service with the mission. In addition to the three mentioned above, two more members of the technical staff left Thailand in disgust during the spring of 1952. Another was discharged with no explanation or hearing from the program administration; and finally, several months ago, the last member of this

group suffered a nervous collapse in Thailand. It is to be hoped that the last vestige of a soundly based agricultural development program for Thailand—the important rice development project that was begun under the Office of Foreign Agricultural Relations—will not, after its long-suffering struggle to get a job done, also experience the fate of other agricultural technical projects.

Why did the Mutual Security Agency force the dissipation of its staff of competent agricultural scientists, and turn some of their responsibilities over to graduate-level assistants? Perhaps the attitude of this kind of administration is best exemplified in an opinion voiced thus by one of the MSA agriculture administrators in Thailand: "The trouble with you technical people is that you're too slow. There's a war going on out here, and we can't wait for you."

There is no doubt that the state agricultural college in a farm state would soon hear in strong language from the state capitol (and from other sources) if it tried to install as dean of the college a boat builder who had no agricultural and no scientific training or experience. But the Mutual Security Agency actually paralleled such an unbelievable maneuver as this in a country which is over 90 per cent agricultural, when it installed the present director of the Thailand agricultural program in November, 1951—in spite of the fact that qualified agricultural leaders available for the post at the time were recommended to the MSA.

It is no wonder, therefore, that an agricultural technical aid staff is faced with a problem in getting on with the job when the administration of their program is supported by the kind of thinking that, for example, desires an immediate nation-wide fertilizer distribution program without experimental data to support such a program; and which does not believe that 120,000 selections of rice can even be kept track of, to say nothing of their culture, and of data on them, in a rice development program.

American scientists throughout the world are trying to do a job on behalf of world progress in their professional fields (such as agriculture) and in the interest of good relations between the American people and the people of other countries. If, as a professional group, the scientists were not wanted in certain areas for the kind of work they can do best, they should not have been recruited at all. A good technical man is suited for little more in the economic aid work than doing a good professional job. Qualified leadership is essential to the success of any technical aid program. The taxpayer has been paying enough to get it, and certainly our foreign relations and economic aid are important enough for it. Party politics and personal gainseeking should have no place in the foreign economic aid efforts of the American government, particularly as they might influence the activities and responsibilities of the technical personnel. The scientists also represent the American people.

J. R. KING

504 N. Fess Ave., Bloomington, Indiana  
July, 1952

## Book Reviews

*The Algae of Illinois.* Lewis Hanford Tiffany and Max Edwin Britton. Chicago: Univ. Chicago Press; London: Cambridge Univ. Press, 1952. 407 pp. Illus. \$10.00.

European botanists have given earnest attention to algal floras since very early times, and, correspondingly, these plants have been adequately dealt with both in Britain and on the continent. Whereas their literature is replete in handbooks and monographic works on algae, these plants, relatively, are poorly represented in the botanical literature of the Western Hemisphere. The increased interest in fresh-water algae in this country during the past decade or so, and the need for published accounts of their taxonomy and ecology, are beginning to be reflected and met. For the second time in two years there has appeared a contribution to our knowledge of fresh-water algae, again from Midwestern America—*The Algae of Illinois*.

Students of the algae will welcome this book for a number of reasons—perhaps the most important one being that the work deals with all seven of the algal phyla (only the Charophyceae in the Chlorophyta and the Chloromonads being disregarded). Of the 888 species treated, a little more than one fourth (238) are diatoms. This group of plants, as biologically intriguing as they are economically important, has been much slighted in North America, and no general account of them has been published in this country since the early work of Francis Wolle. Hence, the present volume answers a great need, and this section of the book might be said to be its most useful contribution.

That Illinois lies mostly outside the region more favorable for desmids in North America is indicated by the inclusion of only 86 species of this very large group. The authors refer, however, to "peat bogs" and "undrained swamps" in the northern part of the state, where desmids undoubtedly abound. Hence, it is to be expected that these algae occur much more prevalently than suggested by their poor representation in the Illinois list.

There is a key to nine classes (subdivisions of algal phyla), which the beginning student will be called on to use in making identifications of unknown species. Each class subsequently treated contains a key to genera, and each genus a key to species. There is no general artificial key to genera, however. Some phycologists will not agree entirely with the authors that "a single key to all genera" has a "value" that is "dubious," nor that it is "fairly easy to recognize the class to which an alga belongs." The beginning student, for whom the book should have the greatest attraction, will need to resort to the initial key to the classes each time he wishes to make an identification. He may experience some difficulty, however, as not a few forms of fresh-water algae do not possess clearly evident

characteristics that place them in this or that phylum. Experienced students and even specialists are occasionally puzzled in attempting properly to assign some genera.

Each species is briefly described with a masterful touch in simplicity and conciseness. Some terms, especially as employed by the phycologist, do not appear in the glossary. Beginners may be bothered at first by the inconsistent use of the terms "chromatophore" and "chloroplast," for the latter term does not occur in the glossary.

The work does not pretend to be critical, and there are few critical remarks, nor are there any new species and varieties described from Illinois. Rather, as is explained, this is the illustrated and descriptive portion of a two-volume catalogue of Illinois algae. It represents the forms observed by the authors and the names that have appeared in the literature. Each form is illustrated, most illustrations being borrowed.

Because of the problem of space, no doubt, ecological notes and references to habitats have been almost entirely omitted—an adjustment which leaves much to be desired.

The determination of an unknown species has been facilitated by what the authors refer to as "convenient signposts." The reader may also find, however, a few dead-end and unmarked streets. These detract but little from the general adequacy and usefulness of the treatment—but the student will not find it possible to key out the genus *Plectonema*, for example (p. 330); also, he will experience some difficulty in keying out species of *Pediastrum* (p. 110), where there is a disagreement between terminology and morphology of various forms. Although the portions of the plate legends giving magnifications of the figures will not be much used, in case they are, the reader should be on guard for discrepancies, as on Plate 12. But these are all very minor problems, easily resolved, especially should the book be used under the supervision of an instructor.

Aquatic organisms are famous for their disrespect of political as well as many natural boundaries, so such a book as this will have a usefulness beyond the implication of its title. It can be predicted that the authors' hopes will be realized and that this volume will stimulate additional studies in American phycology. It is recognizable at once as a fine contribution to our knowledge of algal distribution, and its format should serve as a model for future publications.

There is a good bibliography of reference works, but this list does not include citations to the literature on Illinois algae, especially inasmuch as these appeared in the junior author's, *A Catalog of Illinois Algae* (1944).

G. W. PRESCOTT

Department of Biology, Michigan State College

# The Wiley Bulletin

SCIENCE EDITION

New York

May 22, 1953

## NEW ANDERSON EDITION

### Slants Towards Needs of Non-Science Majors

Most texts in physiological chemistry are written for medical students, but most are too highly technical for general students. These students need and want a text which they can understand, and from which they can get the more important facts of biochemistry. For many years Arthur K. Anderson's "Essentials of Physiological Chemistry" has done this job. Three editions have appeared to date; the last of them was adopted by 100 colleges during its first year. Comments like this show its success:

"(It is) of great value to students who can hardly be expected to be interested in the more purely medical aspects of biochemistry and who are interested in its application to biology, home economics, and related fields." Now a Fourth Edition has appeared, completely modernized.

Among the more important changes are: inclusion of a section on isotopes and of Haworth formulas; rewritten sections on photosynthesis, enzymes, and carbohydrate metabolism; additional material on the structure of starch and cellulose; more material on anti-oxidants; up-to-date sections on chemotherapy and adrenal hormones; and more emphasis on the physiological action of vitamins. The new "Anderson" was published this month with 480 pages at \$5.00.

## VOLUME VII of "ADAMS"

The newest "Organic Reactions," edited by Roger Adams of the University of Illinois, increases the value of the series by including: The Pechmann Reaction; The Skraup Synthesis of Quinolines; Carbon-Carbon Alkylations with Amines and Ammonium Salts; The Von Braun Cyanogen Bromide Reaction; Hydrogenolysis of Benzyl Groups Attached to Oxygen, Nitrogen, or Sulfur; The Nitrosation of Aliphatic Carbon Atoms; and Exoxidation and Hydroxylation of Ethylenic Compounds with Organic Peroxides. Ready in June with approximately 464 pages at a probable price of \$8.50.

## SCIENCE LINKED TO EVERYDAY LIVING

### Unusual Approach Captures Interest

At the right is a study of a transparent plastic model, distinctly showing the lines of stress as the model is bent. Taken from the pages of the new book by Richard Wistar of Mills College, "Man and His Physical Universe: An Integrated Course in Physical Science," it shows a modern application of light in studying the strength of various designs—"nuts, bolts, gears, and even bridges"—as the text explains. This picture illustrates the unusual approach used in this book. It is an example of how the author relates science to life in a striking manner, calculated to hold the interest of the non-science major and to foster a growth of scientific understanding and appreciation.

This illustration is taken from the section on photography in which Professor Wistar demonstrates the principles of light, color, and sound, drawing upon facts from the reader's everyday experience in developing hypotheses and theories to account for physical phenomena. In the same manner, he cuts across several fields of science, integrating them instead of treating each separately, and organizing them around topics of common interest. Factual material is presented first, followed by



Lines of stress revealed by examining transparent model with polarized light. (Polaroid Corp.)

theory, showing how science proceeds from observation to generalization. "Wistar" was published in April with 488 pages and at \$4.75.

## FIRST TRUE INTRODUCTION TO SOLID STATE PHYSICS

Recent years have seen a rapid rise of interest in solid state physics. This, in turn, has presented colleges and universities with the problem of offering adequate instruction in the subject. Professor Charles Kittel of the University of California feels, "... that there should be an introductory or survey course followed by a course in x-ray crystallography and a course in the quantum theory of solids." He, therefore, has written the first and only book on an introductory level covering a large part of the field of solid state physics, "Introduction to Solid State Physics."

This book gives a basic and concise discussion of representative subjects and emphasizes those areas of active re-

search in solids which may be discussed in terms of simple physical models. He keeps the experimental situation in mind with the help of graphs and tables of experimental data, yet holds the book to a basic and short account of the subject.

This new book discusses: ionic crystals, thermal properties, dielectrics, ferroelectrics, paramagnetism, ferromagnetism, antiferromagnetism, superconductivity, electron theory of metals, semi-conductors, transistors, imperfections in crystals, dislocations, etc. Published this month, it will contain 396 pages and is expected to cost \$7.00.

Any of the books on this page may be obtained on approval by writing directly to the publishers:

JOHN WILEY & SONS, Inc., 440 Fourth Avenue, New York 16, N. Y.

May 22, 1953

9

---

## HISTOCHEMISTRY

Theoretical and Applied

by A. G. Everson Pearse

Lecturer in Histochemistry at the  
Postgraduate Medical School (University of London)

*This book is an endeavour to compass the whole of histochemistry as applied to tissue sections. It deals solely, therefore, with the theoretical and practical aspects of Microscopical Histochemistry.*

TECHNICAL APPENDICES TO EACH CHAPTER

530 pages, 109 ill., 4 color plates

\$12.00

---

## CIBA FOUNDATION COLLOQUIA ON ENDOCRINOLOGY

### Volume V

### Bioassay of Anterior Pituitary and Adrenocortical Hormones

#### Contents

*Requirements for Clinically Useful Endocrine Bioassays*

*Thyrotrophic Hormone*

*Gonadotrophins and Prolactin*

*Growth Hormone*

*Adrenocorticotrophic Hormone*

*Adrenocortical Hormone*

224 pages, 53 illustrations

\$6.75

---

## A CIBA FOUNDATION SYMPOSIUM

### VISCERAL CIRCULATION

#### Chairman

Professor J. McMichael  
M.D., F.R.C.P., F.R.S. Ed.

#### Contents

*Visceral Vascular Architecture*

*General Principle of Blood Flow Regulation*

*Regional Blood Flow Regulation*

*Interaction of General and Visceral Circulation*

278 pages, 72 illustrations

\$6.50

---



**LITTLE, BROWN & COMPANY  
BOSTON**

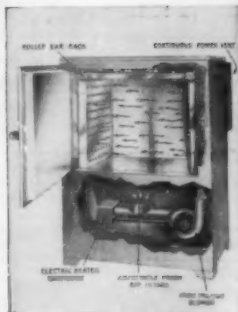
*The Mark of Good Books Since 1837*

---

## Meetings & Conferences

- May 24-28. Scientific Apparatus Makers Association (Annual). The Greenbrier, White Sulphur Springs, W. Va.
- May 25-28. Air Pollution Control Association (Annual). Lord Baltimore Hotel, Baltimore, Md.
- May 25-31. World Congress of Fertility and Sterility. Henry Hudson Hotel, New York.
- May 28-29. American Rheumatism Association. Waldorf-Astoria, New York.
- May 28-31. American College of Chest Physicians. Hotel New Yorker, N. Y.
- May 29-31. Field Conference of Pennsylvania Geologists (Annual). Lafayette College, Easton.
- May 30-31. Society for Investigative Dermatology (Annual). Hotel Belmont Plaza, New York.
- June 1-5. American Medical Association (Annual). New York.
- June 4-6. Canadian Society of Microbiologists. Ontario Agricultural College, Guelph.
- June 5-6. International Congress of Audiology. Leiden.
- June 6-7. International Congress of Bronchology. Utrecht.
- June 7-9. American College of Cardiology (Annual). Hotel Statler, Washington, D. C.
- June 8-13. International Congress of Otorhinolaryngology (5th). Amsterdam.
- June 9-13. American Dermatological Association. Lake Placid Club, Essex County, N. Y.
- June 10-12. Research and Development Associates, Food and Container Institute (Annual). Mayflower Hotel, Washington, D. C.
- June 10-20. General Chemistry and Analytical Chemistry Workshop. Pennsylvania State College.
- June 12-13. Wilson Ornithological Club. Sheboygan, Mich.
- June 12-14. American Medical Technologists (Annual). Hotel Hollenden, Cleveland, Ohio.
- June 14-18. American Society of Medical Technologists (Annual). Brown Hotel, Louisville, Ky.
- June 14-18. Canadian Gas Association. Windsor Hotel, Montreal.
- June 15-17. American Society of Agricultural Engineers (Annual). William Penn Hotel, Pittsburgh.
- June 15-17. American Neurological Association (Annual). Hotel Claridge, Atlantic City, N. J.
- June 15-18. American Society of Mammalogists. American Museum of Natural History, New York.
- June 15-18. American Chemical Society Organic Chemistry Symposium. University of Michigan, Ann Arbor.
- June 15-19. American Society of Civil Engineers. Miami Beach, Fla.
- June 15-19. Canadian Medical Association (Annual). Winnipeg.
- June 15-19. Symposium on Molecular Structure and Spectroscopy. The Ohio State University, Columbus.
- June 15-19. American Institute of Electrical Engineers (Summer General). Chalfonte-Haddon Hall, Atlantic City, N. J.
- June 15-20. American Association for the Advancement of Science. Pacific Division. Santa Barbara, Calif.
- June 15-20. Astronomical Society of the Pacific (with AAAS), Santa Barbara, Calif.
- June 15-20. Western Society of Naturalists (with AAAS). Santa Barbara, Calif.
- June 15-Sept. 4. Gordon Research Conferences, sponsored by AAAS. Colby Junior College, New London, N. H., and New Hampton School, New Hampton, N. H.





### The Reco CHROMATOGRAPHY DRYING OVEN

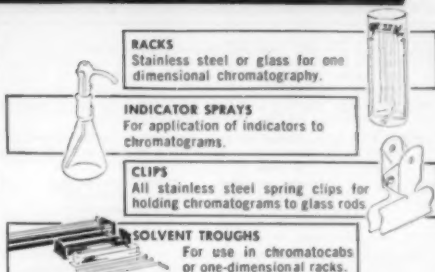
A mechanical convection oven designed for heating paper chromatograms.



### The Reco CHROMATOCAB

For two dimensional paper chromatography, using standard filter paper sheets 18 3/4 by 22 1/2 in., or smaller.

## Paper Partition CHROMATOGRAPHY



#### ALSO AVAILABLE:

Densitometers . . .  
for quantitative determinations on paper  
Ultraviolet Lamps  
Pyrex Disc Chambers  
Micro Pipets  
Desalters

A General Catalogue  
of the RECO Line of  
Paper Partition  
Chromatographic  
Equipment Is  
Available on Request.

## Research Equipment CORPORATION

DEPT. 5 • 1135 THIRD STREET, OAKLAND 20, CALIFORNIA • TWINOAKS 3-0556

## Drying Problem Solved by New Method

New, Niagara "Controlled Humidity" Method provides air at precise conditions of temperature and humidity and permits accurate variations of such conditions at will, in the range of 34°F. to 140°F.; also below 32°. if required.

This Method uses "Hygrol" liquid absorbent to remove moisture from the air directly, saving the cost of refrigeration for dehumidification. Operation is completely and reliably automatic; the absorbent is re-concentrated as fast as it is used.

It is used successfully in drying processes, control of hygroscopic materials, preventing moisture damage to materials or instruments, providing controlled atmosphere for tests and experiments. Units provide capacity ranges from 1000 c. f. m. to 20,000 c. f. m.



Write for Bulletin No. 112; address Niagara Blower Co., Dept. SW, 405 Lexington Avenue, New York 17, N.Y.

## Finest Japanese Optics and Workmanship

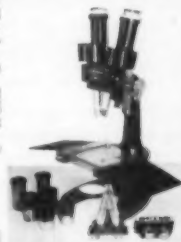
Built to our specifications. Priced far below comparable values. Be assured of a lifetime of excellent performance. Equal to the World's finest optical instruments.

### Greenough Type Stereo-Microscopes (Model UGA)

Used by biological laboratory and students, mineralogical labs, and factories. Excellent for dissecting and mineralogical work. Includes three paired wide-angle eyepieces: 3X, 10X, 15X. Three objectives: 2X, 4X, 10X. Magnification: 10X to 150X. Wt. in carrying case: 11 lbs.

#### Special Introductory offer

**\$195.50**



### Student Microscope (Model UMC-300)

An unusually fine piece of workmanship. Suitable even for making blood count and other laboratory work within the range of its magnification. Stand inclinable to 90°. Objectives: Achromatic: 10X, 20X. Eyepieces: Huygenian: 5X, 15X. Magnification: 50X to 300X. Wt. in case: 5 lbs.

#### Specially Priced

**\$40.00**



Also Inquire About Our Excellent Laboratory Microscope and Binocular Microscope

Prepaid if check or money order accompanies order.

Write for free folder

## UNION MERCANTILE CO., INC.

465 California Street San Francisco, California



# PERSONNEL PLACEMENT

YOUR ad here reaches over 32,000 scientists in the leading educational institutions, industrial laboratories, and research foundations in 77 countries—at a very low cost.

**CLASSIFIED:** 18¢ per word, minimum charge \$3.60. Use of Box Number counts as 10 additional words.

**DISPLAY:** Rates listed below—no charge for Box Number. Monthly invoices will be sent on a charge account basis—provided that satisfactory credit is established.

|                    |                  |
|--------------------|------------------|
| Single insertion   | \$19.50 per inch |
| 7 times in 1 year  | 17.50 per inch   |
| 13 times in 1 year | 16.00 per inch   |
| 26 times in 1 year | 14.00 per inch   |
| 52 times in 1 year | 12.50 per inch   |

**For PROOFS** on display ads, copy must reach SCIENCE 4 weeks before date of issue (Friday of every week).

## POSITIONS WANTED

**Biochemist:** Ph.D.; 1951. Major interest enzymes, proteins, nutrition. Bacteriology minor. Experience enzymes, vitamin E, blood antigens, microbial metabolism. Desires position teaching or institutional, industrial research. Publications. Box 99, SCIENCE. X

**Botanist:** Ph.D.; professor in State University, desires better opportunity for advanced teaching and research. Publications, papers at national meetings. General botany, anatomy, morphology. Box 102, SCIENCE.

**Electron Microscopist:** 7 years supervisory experience with university and research institute, publications. Desires responsibility, growth potential, industrial, applied or research. Box 100, SCIENCE. X

(1) **Food Bacteriologist:** recent Ph.D.; desires industrial research or academic appointment. (2) **Physiologist:** Ph.D.; (Minor: Vertebrate Physiology), qualified teaching; research on tissue culture and cell physiology. Please write Scientific Personnel Service, 122 South Michigan Ave., Chicago. X

**Food Technologist:** Ph.D.; five years teaching experience (Food Technology); four years, director research, food industry. Science Division, Medical Bureau (Burneice Larson, Director), Palmolive Building, Chicago. X

**Genetics teacher:** M.S.; 5 years teaching experience; also training in crops, botany and plant path. Desires position in East or South. P. O. Box 335, Clemson, S. Car. X

**Organic Chemist,** Ph.D., 20 years in pharmaceutical research laboratories, now in Government Laboratory about to be discontinued. Chemistry and isolation of natural compounds, alkaloids, sulfur compounds. Teaching experience, languages. Desires position in academic, industrial research or teaching. Part time considered. N.Y.C. preferred, but will relocate. Box 95, SCIENCE. 5/22

**Pharmacologist,** Ph.D., teaching and research experience including Chemotherapy and tissue respiration studies. Box 93, SCIENCE. 5/22

**Zoology-Comparative Anatomy.** Master's. Man, 31. Four years University teaching. Will continue toward Ph.D. summers. Interested Zoology, Comparative Anatomy, Parasitology. Box 86, SCIENCE. 5/15; 22

## POSITIONS WANTED

**Zoologist;** Ph.D.; 1952. Age 35. Available September. Desires research position. Interested in cancer research; endocrinology; tissue differentiation. Box 103, SCIENCE. 5/22, 29

## POSITIONS OPEN

M.S. in biochemistry to direct Chemistry Laboratory in Chicago Hospital. Practical Hospital experience desirable. To \$6000.00. Box 85, SCIENCE. 5/15; 22

(1) **Botanist;** Ph.D. degree; Major in Plant Physiology or Plant Anatomy; academic appointment; Pacific Coast. (2) **Assistant Editor;** M.S. or Ph.D. in Physiology; excellent opportunity; East. Please write Scientific Personnel Service, 122 S. Michigan Ave., Chicago. X

(a) **Physicians** with scientific training, preferably Ph.D.'s in chemistry, biochemistry or biophysics; university research laboratory expanding facilities; experience or capacity for carrying responsibilities as principal directors of research activities required. (b) **Bacteriologist-Virologist;** research appointment with division of experimental biology; university medical school; West. (c) **Medical Editor** to supervise product literature; well known company; executive ability, medical and nutritional knowledge, skill in medical writing and editing required; \$6000-\$7000. (d) **Director, Clinical Investigation;** key appointment carrying membership, executive committee; internist with degree in one of the sciences preferred. S5-4 Science Division, Medical Bureau (Burneice Larson, Director) Palmolive Bldg., Chicago. X

**Physiologist:** M.S. degree and knowledge French and German essential. Writing and editing. Box 101, SCIENCE. X



## The MARKET PLACE

BOOKS . SERVICES . SUPPLIES . EQUIPMENT

## FOR SALE

**Precision Warburg Apparatus;** circular 20-unit model, used only for short time; in excellent condition, reasonably priced. Box 104, SCIENCE.

## BOOKS

**Your sets and files of scientific journals** are needed by our library and institutional customers. Please send us lists and description of periodical files you are willing to sell at high market prices. Write Dept. A35, J. S. CANNER, INC. Boston 19, Massachusetts

# The MARKET PLACE

BOOKS • SERVICES • SUPPLIES • EQUIPMENT

**CLASSIFIED:** 25¢ per word, minimum charge \$6.00. Use of Box Number counts as 10 additional words. Correct payment to SCIENCE must accompany ad.

**DISPLAY:** Rates listed below—no charge for Box Number. Monthly invoices will be sent on a charge account basis—provided that satisfactory credit is established.

|                    |                  |
|--------------------|------------------|
| Single insertion   | \$19.50 per inch |
| 7 times in 1 year  | 17.50 per inch   |
| 13 times in 1 year | 16.00 per inch   |
| 26 times in 1 year | 14.00 per inch   |
| 52 times in 1 year | 12.50 per inch   |

For PROOFS on display ads, copy must reach SCIENCE 4 weeks before date of issue (Friday of every week).

## BOOKS

**SCIENTIFIC BOOKS and PERIODICALS WANTED.**  
Complete libraries—Sets and runs—Single titles  
Also, please send us your want lists.

**STECHELT - HAFNER, INC.**  
31 East 10th St., New York 3

**WANTED TO PURCHASE . . .**  
**SCIENTIFIC PERIODICALS and BOOKS**

Sets and runs, foreign and domestic. Entire libraries and smaller collections wanted.

**WALTER J. JOHNSON • 125 East 23rd St., New York 10, N. Y.**

## SUPPLIES AND EQUIPMENT

### • HYPOPHYSECTOMIZED RATS

Shipped to all points via Air Express  
For further information write

**HORMONE ASSAY LABORATORIES, Inc. • 8159 South Spaulding Ave. Chicago 29, Ill.**

**HORMONE DETERMINATIONS**  
F.S.H.—ESTROGEN—PREGNANDIOL—  
17 KETOSTEROIDS

**STARKMAN Biological Laboratory • 461 Bloor Street West Toronto, Ont., Canada**  
Literature on request

### Self-Adhesive Labels

993 994 995 996 997

Consecutively  
Numbered

For numbering Samples Consecutively.

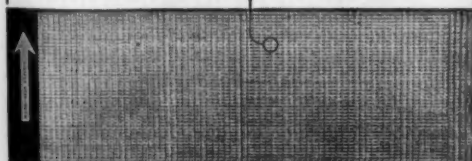
Easy to Use—Speedy—Error-Proof.

Set 1 to 1000, in dispenser . . . . . \$6.50  
Extra Sets of 1000 . . . . . Each . . . . . \$4.00  
5 Sets — \$18.00 Samples LS on Request

**R. P. Cargille Laboratories, Inc.**

117 Liberty St. New York 6, N. Y.

## MICRO-VISOR



Green Glass Slide with 3750 Numbered Rectangles to find same field again or to mark for photography.

\$10.00

Order From:

**ERIC SOBOTKA CO.** 102 West 42nd St. New York 36, N. Y.



## SUPPLIES AND EQUIPMENT

**White Albino Rats:** Wistar strain, established since 1939. We are breeders, not dealers. Our plant is open for inspection at all times. Edgewood Breeding Farm, R.F.D. #2, Rahway, N. J. 5/22, 29

**NINHYDRIN  
L-GLUTAMINE**

**PIERCE  
CHEMICAL CO.**  
P. O. Box 117  
Rockford, Ill.

# RATS

Long-Evans (PAF)  
Wistar (PAF)

Bred for Research Work by Research Workers  
**PACIFIC ANIMAL FARMS**  
2457 Fletcher Dr., Los Angeles 39, Calif.

## All AMINO ACIDS

natural, synthetic, unnatural,  
Rare Sugars, Biochemical Products, Reagents, New  
Pharmaceuticals in stock. Write or phone PLaza  
7-8171 for complete price list.  
**BIOS LABORATORIES, INC.** 17 West 60th Street,  
New York 23, N. Y.

### β-Glucuronidase (Calf Spleen)

for urinary cortical assay† 25,000 Fishman Units per gram\*  
(in ammonium sulfate).

Dry powder—stable at room temperature.

\*Fishman, W. H., et al., J. B. C. 173, 449 (1948)

†Corcoran, A. C., et al., J. Lab. & Clin. Med. 36, 297 (1950)

### Uricase

Derived from kidney. Standardized; 5 mg. catalyzes conversion one micromole uric acid to non-chromogenic end products in 90 minutes at 45° C\*.

\*Wolfson, W. Q., et al., J. Expt. Med. 92, 121-27 (1950)

Also available—Cytochrome C, vials of 50 mg. (10cc).  
Raw Liver, Duodenum, Stomach, and other tissue preparations for enzyme study.

Write for catalog.

**VIOLIN Laboratories • Monticello, Illinois**

## Lanco STAINLESS STEEL BEAKERS

1. RUGGED
2. TOUGH
3. POUROUT SPOUT
4. EYE APPEAL
5. EVEN HEAT DISTRIBUTION



(GRIFFIN  
LOW FORM)

|         | No. 21-43-S |        |          |
|---------|-------------|--------|----------|
| Size    | Approx. Wt. | Each   | Doz. Lat |
| 50 ml   | 37 gm       | \$1.20 | \$1.10   |
| 125 ml  | 66 gm       | 1.55   | 1.40     |
| 250 ml  | 126 gm      | 2.10   | 1.90     |
| 600 ml  | 242 gm      | 2.75   | 2.50     |
| 1200 ml | 325 gm      | 3.40   | 3.05     |
| 2000 ml | 446 gm      | 4.20   | 3.80     |
| 3000 ml | 586 gm      | 4.75   | 4.25     |
| 4000 ml | 691 gm      | 5.45   | 4.90     |

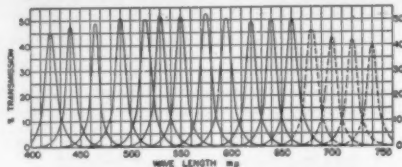
**ARTHUR S. LAPINE and COMPANY** **LAPINE** 6001 S. KNOX AVE., CHICAGO 29, ILL.

LABORATORY SUPPLIES EQUIPMENT

## G.A.B. Interference Filters

(Made in Switzerland)

for isolating narrow spectral bands



Spectral Range: 400-900 millimicrons

Spectral Width: 12-15 mμ, Transm.: 45-50%

Size: 2" x 2". Other sizes on order.

For

Flame Photometry and Color Densitometry  
Microscopy and Photomicrography  
Colorimetry and Fluorimetry

also in reflectometry, light scattering measurements, microcolorimetry, refractometry, polarimetry, and in all other fields requiring monochromatic light in the visible and near-infrared range.

Write for Bulletin #180 to

**PHOTOVOLT CORP.**

95 Madison Ave.

New York 16, N. Y.



## AMINO ACIDS O.S.

(OPTICALLY STANDARDIZED)

IMMEDIATELY AVAILABLE  
L- and D-Isomers

For highest precision in chromatography, microbiological assay, nutrition, syntheses, and all other exacting requirements for amino acids.

Write for our new Price List showing specific rotation ranges for our O.S. (Optically Standardized) grade.

Every package label gives the exact rotation of the lot shipped. Per cent nitrogen is specified to be within  $\pm 0.1$  of theoretical value.

Specify Schwarz for assurance of highest purity in biochemicals.

BL290C

**SCHWARZ LABORATORIES, INC.**

230 WASHINGTON STREET, MOUNT VERNON, N. Y.

## The MARKET PLACE

BOOKS • SERVICES • SUPPLIES • EQUIPMENT

### SUPPLIES AND EQUIPMENT

### 1953 DIGEST

#### CARGILLE LABORATORY AIDS

Just write for Digest 5M

R. P. CARGILLE LABORATORIES, INC.  
117 Liberty St. • New York 6, N. Y.

### PROFESSIONAL SERVICES



#### FOOD RESEARCH LABORATORIES, INC.

Founded 1922

Philip B. Hawk, Ph.D., President

Bernard L. Oser, Ph.D., Director

Research • Analysis • Consultation

Biological, Nutritional, Toxicological Studies

for the Food, Drug and Allied Industries

48-14 33rd Street, Long Island City 1, N.Y.

• RESEARCH

• CONSULTATION

• ANALYSES

### LOOKING FOR A PUBLISHER?

Write for Free Booklet SC telling how we can publish your book. All subjects considered. New authors welcome.  
VANTAGE PRESS, Inc. • 120 W. 31 St., New York 1.  
In Calif.: 6356 Hollywood Blvd., Hollywood 28

SINCE 1870

Food Ingredient & New Drug Studies



#### DIGITALIS ASSAYS

**LaWall & Harrisson**

Div. S, 1921 Walnut St., Philadelphia 3, Pa.

Bacteriologists  
Chemists • Pharmacologists

ANALYSES • CONSULTATION • RESEARCH

### SPECIAL GLASS APPARATUS

Our glass blowing department is available for special scientific and technical glass apparatus made to specifications and drawings. Inquiries invited. Estimates furnished.

**E. MACHLETT & SON**  
218 East 23rd St. New York 10, N. Y.



### LABORATORY SERVICES

Project research and consultation in  
Biochemistry, Chemistry, Bacteriology  
and Entomology

Screening of compounds for insecticidal, fungicidal and bactericidal properties • Biological evaluation and chemical determination of insecticides • Peet-Grady and C.S.M.A. aerosol tests • Warfarin assays

Write for price schedule

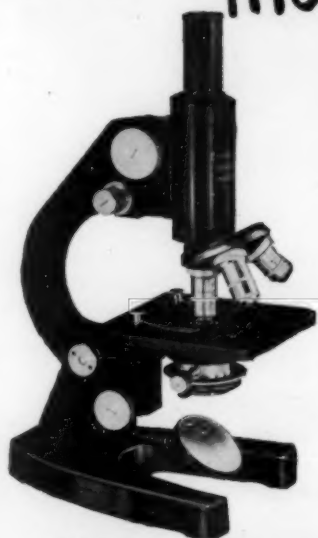
**WISCONSIN ALUMNI RESEARCH FOUNDATION**

P. O. BOX 2059 • MADISON 1, WISCONSIN

Buy your *Leitz*<sup>®</sup>

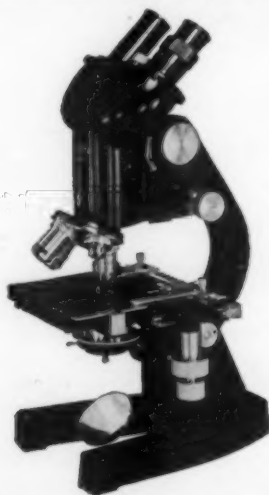
# Medical Microscope now—

use it for a *lifetime* of practice



Leitz Monocular Microscope,  
Model GO-L 20/92K Built-in Illumination

Leitz Interchangeable Inclined  
Binocular Microscope, Model BS 48/92K



Doctors and scientists the world over still use the Leitz Microscopes they bought and used in school. They've found them more durable, and infinitely more accurate than any other make they've tried. Take a tip from these men.

*Make a lifetime investment.* Buy a Leitz microscope now. Ask your dealer for a look at the Leitz Medical Microscope and COMPARE it with other instruments... SEE the difference in workmanship, more detailed image and ease of operation.

See us June 1st through 5th at American Medical Association Convention at Grand Central Palace, New York, New York.

For details write Dept. SC. **E. LEITZ, Inc.**, 468 Fourth Avenue, New York 16, N. Y.

LEITZ MICROSCOPES • SCIENTIFIC INSTRUMENTS • BINOCULARS  
LEICA CAMERAS AND ACCESSORIES

**Pipes  
that  
grew  
without  
getting  
bigger**



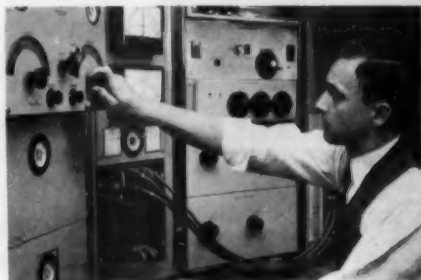
Cross-section of coaxial cable. To triple capacity, Bell Laboratories and Western Electric engineers had to make 1000 amplifiers work perfectly in tandem . . . feed repeater power along the same cable that carries messages . . . put signals on and off the line at numerous cities along the route without distortion.

Pencil-size pipes carry telephone messages and television across country through the Bell System's coaxial cable. Once, each pipe could carry 600 voices, or one television program. Now it can carry 1800 voices, or 600 voices *plus* a broadcast quality television program.

Yet the pipes aren't any larger. They are being made into triple-duty voiceways by new repeaters, new terminal equipment and other transmission advances developed by Bell Laboratories engineers.

The conversion expense is less than the cost of laying extra coaxial cables. But it calls for highly refined manufacturing procedures, made possible only by close co-operation of Bell Laboratories and Western Electric, manufacturing unit of the Bell System.

In improving the coaxial cable system, they created more than 20 years ago, engineers at Bell Telephone Laboratories devised a new way to give America still better telephone service, while the cost stays low.



Laboratories engineer tests new triple-duty coaxial system. It marks the first time that telephone conversations and television can travel through the same pipes at the same time. With a wider frequency band being transmitted, big problem was to eliminate interference between the two types of signals.



**BELL TELEPHONE  
LABORATORIES**

Improving telephone service for America provides careers for creative men in scientific and technical fields



